**PATENT APPLICATION**

***IN THE UNITED STATES PATENT AND TRADEMARK OFFICE***

In re Application of: **Praveen SHARMA et al.** Conf. No.: **8084**

Appln. No.: **10/727,576** Group Art Unit: **1634**

Filed: **December 05, 2003** Examiner: **Juliet C. Switzer**

For: **METHOD OF PREPARING A STANDARD DIAGNOSTIC GENE TRANSCRIPT PATTERN**

DECLARATION UNDER 37 C.F.R. § 1.132

Assistant Commissioner of Patents
P.O. Box. 1450
Alexandria, Virginia 22313-1450

I, Dr James Mackay, a British citizen of Suite 4, Claridge House, 32 Davies Street, London, W1K 4ND;

declare as follows:

1. I am an accredited medical oncologist on the Specialist register of the General Medical Council, and I am a Fellow of the Royal Colleges of Physicians of Edinburgh and of London. I am a Consultant Clinical Genetic Oncologist in the research department of Genetics, Evolution and Environment in University College London. I see patients as a Consultant Physician in The London Clinic, The London Oncology Clinic and The London Breast Clinic. My specialist areas of academic research and clinical practice are in cancer genetics, the management of familial cancer, screening for breast and ovarian cancer and the introduction of innovative laboratory technology into the clinical care of the cancer patient, and the diagnosis of early cancer. A copy of my curriculum vitae is attached (Annex 1).

2. I have reviewed US 6,190,857 (Ralph *et al*) and have been asked to provide my opinion on how I would have interpreted the teachings and suggestions in that document in April 1997. In particular I have been directed to the teaching in column 9, line 66 to column 10, line 6 which refers to applying the described method to discover disease markers for any disease state that affects peripheral blood leukocytes such as metastatic or organ defined cancer.

3. I believe that the comments set forth in column 9 referring to organ defined cancer are based on the results obtained with organ confined prostate cancer samples, reported in column 6, from line 61 which I assume relates to the results presented in column 94, line 43 to column 95, line 17. The passage in column 5 states that the method allows the identification of patients with organ confined prostate cancer (relative to those with benign prostatic hyperplasia) using analysis of PSA and IL-8 gene products and this is the conclusion of the results presented in columns 94 and 95.

4. In the results presented in columns 94 and 95 patients with stage A to C organ confined prostate cancer were examined. A significant proportion of those patients would have already developed metastatic disease. It was well known at the time that patients who have prostate cancer which appears to be confined to the organ (that is the prostate) often have already developed metastatic disease, which may indeed be low volume disease at initial presentation but which declares itself with time. Newman (1996, Radiologic Technology, Volume 68, No. 1, p39-64, enclosed as Annex 2) reports that up to 25% of patients with stage A prostate cancer have early evidence of metastatic disease when examined with sensitive molecular biological techniques (page 50, left hand column). Seiden *et al* (1994, Journal of Clinical Oncology, Volume 12, No. 12, p2634-2639, enclosed as Annex 3) confirms that some localized prostate cancer stages (stages A to C) have metastasized, see Table 3 on page 2636 and page 2637, left hand column, bottom 5 lines. Furthermore, in the progression to metastasis, cancer cells begin to exhibit intrinsic properties which may be used as clinically detectable indicators of cells which have metastatic potential. Cells with metastatic potential are thus subtlety different to pre-metastatic cancer cells and can be expected to have released debris or cellular components which would ultimately appear in the peripheral blood system even before metastasis has occurred.

5. I believe that the skilled person reading the work of Ralph *et al*, would understand that the method described by Ralph *et al* was based on release of cancer cells, their debris or cellular components into the peripheral blood system resulting in direct interaction between cancer cells (or their debris or cellular components) and cells of the peripheral blood system, thereby affecting gene expression in those blood cells. Ralph *et al* states that direct contact between the sample to be analyzed and the diseased cells is necessary for performance of the method. This is evident from column 47, line 17 which refers to the type of sample to be used. There it is stated that any biological fluid may be used that comes into contact with diseased tissues. It is further stated in column 52, lines 1-4, that in the case of early stages of the disease states, the immune response may be localized, e.g. to lymph nodes immediately surrounding a metastasizing tumour.

6. The Ralph *et al* method would thus, at most, be expected to allow the subtle detection of the very earliest signs of metastatic disease in cancers in which cancer cells or their debris or cellular components have been released and are in direct contact with blood cells. Organ confined prostate cancers would fall within this group in view of the fact that most organ confined prostate cancers have already released cells, their debris or cellular components into the bloodstream, i.e. have cells with metastatic potential or are metastatic in nature.

7. However, disease presentation in prostate and breast cancer is different. Organ confined breast cancer is not equivalent to organ confined prostate cancer. Whereas in

prostate cancer the cells of most organ confined cancers already exhibit metastatic potential or the cancer has already progressed to metastatic disease, In breast cancer we see a different spectrum of disease presentation. The vast majority of very small breast cancers detected by regular mammographic screening never develop metastatic disease. The % of patients who have involved lymph nodes at diagnosis is low. Only a few patients with small node negative tumours at diagnosis will go on to develop metastases within 5 years of diagnosis, and these patients are considered to have particularly biologically aggressive disease at diagnosis. However, in the clinical care of breast patients, cancer confined to the organ (that is the breast) covers a very wide spectrum of disease from early to very advanced.

8. In prostate cancer, many organ confined cancers, even some apparently early stage prostate cancers would have developed to the metastatic stage, i.e. the cells display metastatic potential or the disease is metastatic. In contrast, in breast cancer, few organ confined cancers would have even developed cells with metastatic potential and those that had would be at the advanced stage. Very early stage breast cancer would not present any cells with metastatic potential.

9. As mentioned above in paragraph 5, it is my view that the skilled person would believe that the Ralph *et al* method could only be extended to cancers in which contact between the cancer cells (or their debris or cellular components) and peripheral blood cells occurred. The method is therefore applicable once the first signs of cells with metastatic potential or metastasis appear. Since organ confined breast cancer which is still at an early stage does not have cells with metastatic potential, one would not expect that the peripheral blood cells could come into contact with metastatic breast cancer cells or their debris or cellular components at that stage. As a consequence, I am of the opinion that the skilled person when reading Ralph *et al* would not consider applying the teaching to the analysis of very early stage breast cancer and would not expect that such a method would be successful. The skilled person would expect that cancers to be detected would need to have cells exhibiting metastatic potential or have become metastatic. This would not include very early stage breast cancer since such cancers do not have cells exhibiting metastatic potential.

10. The passage bridging columns 9 and 10 would have been interpreted in that context. The reference to applying the method to organ defined cancer would necessarily have been interpreted at the time of the invention as applying to organ defined cancers in which the cells being analyzed had had direct contact with cancer cells (or their debris or cellular components). As a consequence, if peripheral blood were to be used, the method could only be applied to organ defined cancers in which peripheral blood cells had had direct contact with cancer cells or their debris or cellular components. As it was known that very early stage breast cancer does not exhibit any signs of metastatic potential or metastasis, the skilled person would assume that the reference to organ defined cancer in columns 9 and 10 could not apply to very early stage breast cancer and would have had no reason to apply the method to analysis of such cancers. In view of the teaching there would have been no expectation that applying the method to very early stage breast cancer would be successful.

11. The other cited document by Lukas *et al* would not alter this conclusion. Lukas *et al* looks only at the tumour cells themselves and provides no information on whether the expression of peripheral blood cells would be affected.

12. In 1997 it was surprising even taking into account the teaching of Ralph *et al*, that very early stage breast cancer could be diagnosed by analyzing peripheral blood samples. Ralph *et al* was clearly of the view that direct contact between cancer cells and blood cells was necessary to alter gene expression in those blood cells. In contrast, the inventors of the instant application have found that no such direct contact is required and they have provided, for the first time, a diagnostic method for very early stage breast cancer in which gene expression of a peripheral blood sample is analyzed.

13. I further declare that all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true, and that these statements were made with the knowledge that wilful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Codes, and that such wilful false statements may jeopardize the validity of the application and any patent issuing thereon.

James Mackay ........................ 25th March 2009 ........................
Dr James Mackay | Date

# ANNEX 1

Curriculum Vitae of Dr James Mackay

# Dr James Mackay
# MA, MD, FRCP, FRCPE
# Consultant Clinical Genetic Oncologist

Research Department of Genetics, Evolution and Environment
University College London
Wolfson House
4 Stephenson Way
London NW1 2HE
j.mackay@ucl.ac.uk

## Profile

- Accredited medical oncologist (1995)
- Experienced in setting up and running clinical cancer genetics services
- Clinical Adviser to the Cancer Division of the MRC Clinical Trials Unit
- Member of the Committee on Medical Aspects of Radiation in the Environment appointed by Chief Medical Officer; (2005-2007)
- Joint P.I. of a study collecting family history details and ovarian screening data from 5,000 well women with a family history of ovarian cancer (UK FOCSS)
- P.I. of an evaluation of mammographic services in women under 50 with a family history of breast cancer; collecting family history information and mammographic data from a cohort of 10,000 well women aged between 40 and 44 (the FH 01 study)
- P.I. on the Genetic Breast Cancer Trial; The first randomised prospective trial of cytotoxic chemotherapy based on inherited genotype in the world (2004-2007)
- Clinical lead on the University of Manchester/UCL Clinical e-science Framework Project funded by the MRC (C-LEF services)
- Clinical lead on the Cancerbackup cancer genetics project to provide high class patient information on genetic testing and family history in clinical management
- Chair of the Great Ormond Street Hospital e-medicine committee (2004-2006)
- Member of the Scientific Advisory Group of the CRUK and UCL Cancer Trials Centre
- Member of the NCRI Breast Cancer Clinical Studies Group (2002-2007)
- Member of the NICE Familial Breast Cancer Management Guidelines Development Group
- Member of the Educational Faculty of the European Society of Medical Oncology
- ESMO Faculty Coordinator for the Cancer Genetics Faculty Group
- Chair of the steering committee for the Cancerbackup genetics project
- Expert Clinical Advisor to Breakthrough Breast Cancer
- Member of the National Clinical Advisory Board of Maggie's Caring Cancer Centre
- Visiting Professor in the Institute of Communication and Health at the University of Lugano.

## Education Summary

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|---|---|
| 1980 **BA** | Trinity College, University of Cambridge |
| 1983 **MB, ChB** | University of Edinburgh Medical School |
| 1984 **MA** | Trinity College, University of Cambridge |
| 1989 **MD** | University of Edinburgh Medical School |
| 1999 **FRCPE** | The Royal College of Physicians of Edinburgh |
| 2000 **FRCP** | The Royal College of Physicians of London |

## Current Position

Consultant Clinical Genetic Oncologist

**The London Oncology Clinic**
95 Harley Street, London
**The London Clinic**
119 Harley Street, London
**The London Breast Clinic**
108 Harley Street, London
**The Princess Grace Hospital**
42-52 Nottingham Place, London
**Nuffield Hospital**
Tunbridge Wells, Kent
**Nuffield Hospital**
Cheltenham
**Nuffield Hospital**
Cambridge
**Ramsay Yorkshire Clinic**
Bingley, West Yorkshire
**Ramsay West Midlands Clinic**
Halesowen, West Midlands
**Ramsay Springfield Hospital**
Chelmsford, Essex
**BMI Alexandra Hospital**
Cheadle, Cheshire
**Spire Lea Hospital**
Cambridge

Honorary Senior Lecturer

**Research Department of Genetics, Evolution and Environment**
**UCL** Wolfson House, 4 Stephenson Way, London

## Career History

**June 2001 – July 2006**

- Consultant Clinical Genetic Oncologist, The North East Thames Clinical Genetics Service, The Genetics Unit, Great Ormond Street Hospital NHS Trust, London
- Hon. Senior Lecturer, The Clinical and Molecular Genetics Unit, The Institute of Child Health, University College London
- Hon. Senior Lecturer, The Department of Medicine, Royal Free and University College Medical School, University College London
- Hon. Senior Lecturer, The Department of Oncology, Royal Free and University College Medical School, University College London

- Hon. Senior Lecturer, The Centre for Health Informatics and Multi-professional Education, University College London
- Hon. Consultant Clinical Genetic Oncologist, The University College London Hospitals NHS Trust, London
- Hon. Consultant Oncologist, The Royal Free Hospital NHS Trust
- Hon. Senior Lecturer, Division of Haematology and Oncology, Queen Mary and Westfield College, The University of London
- Hon. Consultant Oncologist, The Department of Oncology, St Bartholomew's and the Royal London Hospitals NHS Trust

I was recruited to the North East Thames Clinical Genetics Service in June 2001 to set up cancer genetics services in the region. I worked closely with colleagues from four cancer networks to establish these services. In April 2004, I presented a report on the programme of investment, which I had managed, totalling new recurrent funding of £480,000 over three years, to the Regional Specialist Commissioning Group. This report was well received.
In September 2002, I was appointed as an Honorary Senior lecturer in the Institute of Child Health, spending half my time on academic projects within University College London. I then initiated and developed remote cancer genetic clinical services using live tele-conferencing technology. This became the first regular remote cancer genetics clinic in the world, and has helped shape the e-health policy for Great Ormond Street Hospital NHS Trust.

**February 1996 – May 2001**

- Senior Research Associate in the University of Cambridge School of Clinical Medicine
- Hon Consultant Clinical Oncologist, Addenbrooke's Hospital NHS Trust, Cambridge
- Hon. Consultant in Cancer Genetics, The Gynaecological Division of the Royal Marsden Hospital NHS Trust, London
- Hon Consultant in Cancer Genetics, The Royal Hospitals NHS Trust St Bartholomew's Hospital, London
- Hon Consultant in Cancer Genetics, Derby Cancer Centre, Derby

I held this CRC funded position for five years. In close collaboration with colleagues in public health, breast surgery, genetics, oncology and radiology, I originated and disseminated a policy for the management of women with a family history of breast cancer. These ideas have gained wide acceptance.
With the assistance of one clinical nurse specialist I set up and ran the regional cancer genetics service attracting referrals from a region of 2.1/2 million population and extra referrals on a supra-regional basis.
I also ran with the clinical nurse specialist the Cambridge Familial Breast Cancer Clinic, used as a model for other familial clinics throughout the region. I acted as expert advisor to a network of familial breast clinics run by breast surgeons throughout the region and beyond.
In collaboration with Mr Robin Crawford, Consultant Gynaecologist, I set up and ran the Addenbrooke's Familial Ovarian Cancer Clinic.

**October 1993 - January 1996**

- I.C.R.F. Clinical Research Fellow and Honorary Senior Registrar in the Department of Medical Oncology, Western General Hospital, Edinburgh (JCHMT Accreditation No: 26/150).

I was responsible, with one Consultant, for all adjuvant and advanced disease chemotherapy in the Edinburgh Breast Unit, until January 1995. This is the largest Breast Unit in the United Kingdom, seeing 450 new cases of breast cancers per year and specialising in myeloablative chemotherapy with peripheral blood stem cell support in young women with aggressive metastatic disease.

I then became responsible with 3 Consultants for the South East of Scotland Department of Medical Oncology. This busy supraregional service treated patients with a wide variety of tumours, specialising in new drug development for the treatment of ovarian, small cell and non small cell lung cancers, lymphomas and teratomas.
I was on a 1:6 on call rota providing out of hours cover for the Department of Medical Oncology, Oncology patients in the Edinburgh Breast Unit and the Department of Haematology in the Western General Hospital.

I had a part-time attachment to the South East of Scotland Clinical Genetics Services, with responsibility of the Cancer Genetics Service. The Edinburgh Familial Breast Cancer Clinic sees 750 new referrals per year and the Familial Ovarian Cancer Clinic see 150 new referrals per year. In my general cancer genetics clinic I saw referrals with a family history of polyposis coli, non-polyposis colonic cancer and other less common familial cancer syndromes.

## Registrar Posts

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|---|---|
| **October 1992 - September 1993** | Western General Hospital, Edinburgh Department of Medical Oncology, Professor J Smyth and Dr R C F Leonard |
| **August 1992 - September 1992** | The Royal Berkshire Hospital, Reading General Medicine & Gastroenterology, Dr M Myzor |
| **February 1992 - July 1992** | The Royal Berkshire Hospital, Reading General Medicine, Diabetes & Endocrinology, Dr H Simpson |
| **August 1991 - January 1992** | The Royal Berkshire Hospital, Reading General Medicine & Nephrology, Dr R Naik |
| **February 1991 - July 1991** | Battle Hospital, ReadingNeurology and Acute Care of the ElderlyDr N Hyman and Dr R Allen-Narker |
| **August 1990 - January 1991** | Western General Hospital, EdinburghDepartment of Radiation OncologyDr A Gregor and Dr G Howard |
| **December 1989 - July 1990** | Western General Hospital, EdinburghDepartment of Medical OncologyProfessor J F Smyth and Dr R C F Leonard |
| **August 1985 - July 1988** | Royal Infirmary of EdinburghAccident & Emergency DepartmentDr K Little |

## Senior House Officer Posts

| | |
|---|---|
| **February 1985 - July 1985** | Royal Infirmary of EdinburghAccident and Emergency DepartmentDr K Little |
| **August 1984 - January 1985** | Royal Hospital for Sick Children, EdinburghPaediatric SurgeryMr W H Bisset and Mr G A McKinley |

## House Officer Posts

| | |
|---|---|
| SURGERY | |
| **February 1984 - July 1985** | Royal Infirmary of Edinburgh, Wards 7/8Professor Sir Patrick Forrest |
| MEDICINE | |
| **August 1983 - January 1984** | Eastern General Hospital, EdinburghDr J Nimmo and Dr R J Kellett |

## Major Current Grants

**CRUK National Familial Ovarian Cancer Screening Study.** Joint principal investigator with Professor Ian Jacobs, Director, Institute of Womens Health, UCL, London. CRUK contribution £549,763; NCI (USA) contribution £477,621; NHS R and D Direct Subvention £500,000Total project value £2.5 million

**The NHS R and D Health Technology Assessment programme.** Principal Investigator: A multicentre evaluation of mammographic surveillance services in women under 50 with a family history of breast cancer. NHS R and D HTA programme start-up grant £570,000; continuation grant £435,376CRUK Psycho-social and Education Research Committee programme grant to the University of Oxford £330,000;Total project value £3.7 million

**The CancerBACUP Cancer Genetics project.** Joint Principal applicant. Department of Health Section 64 contribution £134,000; contribution from WNPCC wind-up £ 42,000;Total project value £176,000

**CRUK UCL Cancer Trials Centre.** With Ledermann J, Sainsbury R, Linch D and Begent R. 5 year programme funding of £2.25 million

**The Breakthrough and Cancer Research UK Genetic Breast Cancer Trial.** Joint P.I. with Parmar M and Tutt A.CRUK and MRC Clinical Trial Advisory and Adoption Committee £295,810

**London Genetics Ltd.** Clinical Lead with Professor Steve Smith, Pricipal of the Medical School, Imperial College London.The Department of Trade and Industry and the London Development Agency. £ 2.0 million

**CLEF services.** Clinical lead with Rector A, with Ingram D, Kalra D and Singleton P.MRC and Research Councils UK Clinical e-science programme. £2.9 million

**Project OPERA.** An on-line risk assessment tool providing personalised inherited breast cancer risk information, and information on available management options to the general public. Clinical genetic lead, with Pithers A, Schulz P, Paolini P and CancerBACUP. The King's Fund. £50,000

## Previous Grants held

**NHS National Cancer Research and Development Programme.** Evaluating services for women with familial risk of breast cancer with Kinmonth, Todd, Marteau, Bobrow March 1997. 14 months £67,500.

**NHS Executive Anglia and Oxford Regional Research and Development Fund.** Psychological impact of genetic testing for hereditary breast and ovarian cancer with Marteau, Michie, Broadstock. May 1997. 22 months £50,988.

**Wellbeing and the Royal College of Obstetrics and Gynaecologists.** The psychosocial implication of prophylactic oophorectomy with Halliwell, Gore, Richards, Jacobs. May 1997. 2 years £62,950.

**NHS Executive Anglia and Oxford Regional Research and Development Fund.** Assessment of a questionnaire to identify patients at increased risk of common familial cancers in primary care, with Leggatt and Yates. Sept 1997. 12 months £37,737

The **British Council.** Finnish-British academic research collaboration scheme with Mannermaa, Kataja and Ponder. Collaborative travel grant £1,500. 1998

**CRC Clinical Trials Committee**; IBIS RAZOR: a pilot randomised trial of Zoladex plus raloxifene plus screening versus screening alone for the prevention of breast cancer in premenopausal women at high genetic risk, with Dowsett M, Cuzick J. April 2000. £60,987

## Peer Reviewed Scientific Publications


**Mackay J**, MacAreavey D and Robertson CE. Prolonged mechanical cardiopulmonary resuscitation. Br J of A and E Medicine, 1987. 2: 15

**Mackay J**, Elder PA, Porteous DJ, Steel CM, Hawkins RA, Going JJ and Chetty U. Partial deletion of chromosome 11p in breast cancer correlates with size of primary tumour and oestrogen receptor level. Br J of Cancer, 1988. 58: 710-714

**Mackay J**, Elder PA, Steel CM, Forrest APM and Evans HJ. Allele loss on short arm of chromosome 17 in breast cancers. The Lancet, 1988. (11): 1384-1385

**Mackay J**, Thompson AM, Coles C and Steel CM. Molecular lesions in breast cancer. Int J of Cancer, 1990, suppl. 28-36

Coles C, Thompson AM, Elder PA, **Mackay J**, et al. Evidence implicating at least two genes on chromosome 17p in breast carcinogenesis. The Lancet, 1990, (11) 761-763.

Porter DE, Spowart G, Morrison H, Mitchell M, Thompson AM, **Mackay J**, Steel CM, et al. Cytogenetic analysis of DNA stability in stimulated cells in chromosome 17q breast cancer gene carriers and non-gene carriers. Br J of Cancer, 1993, 67 (suppl.XX) 12.

Wall LC, **Mackay J**, Leonard RCF. Unusual presentations of malignancy. Medicine 1995, 23,11, 441-442.

Gabra H, Langdon SP, Watson JEV, **Mackay J**, Steel CM, Leonard RCF, Smyth JF. Loss of heterozygosity at 11q22 correlates with low progesterone receptor content in epithelial ovarian cancer. Clinical Cancer Research 1995, I, 945-953.

Campbell H, **Mackay J**, Porteous M. The future of breast and ovarian cancer clinics. BMJ 1995, 311, 1584-1585.

Gabra H, Watson JEV, Taylor K, **Mackay J**, Leonard RCF, Steel CM, Porteous DJ, Smith JF. Definition and refinement of a new region of loss of heterozygosity at 11q23.3-q24.3 in epithelian ovarian cancer associated with poor prognosis. Cancer Res, 1996, 56, 950-954.

Gayther SA, Harrington P, Russell P, Garkavtseva RF, Ponder BAJ and The UKCCCR Familial Ovarian Cancer Study Group. Rapid detection of regionally clustered germline BRCA1 mutations by heteroduplex analysis. Am. J. Hum. Genet. 1996, 58, 451-456.

Mullen P, Miller WR, **Mackay J**, Fitzpatrick DR, Langdon SP, Warner JP. BRCA1 5382insC mutation in sporadic and familial breast and ovarian carcinoma in Scotland. Br J of Cancer, 1997, 75: 1377-1380

**Mackay J**, Cameron DA, Gardiner J, Leonard T, Lee LE, Leonard RCF. A pilot study of infusional CMF (CMF-infl): Active and well tolerated in breast cancer. Annals of Oncology, 1996, 7: 409-411.

Cummings J, Langdon SP, Ritchie AA, Burns DJ, **Mackay J**, Stockman P. Pharmacokinetics, metabolism and tumour disposition of 8-chloroadenosine 3', 5'- monophosphate in breast cancer patients and xenograft bearing miceAnnals of Oncology, 1996, 7: 291-296

Cameron DA, Craig J, Gabra H, Lee L, **Mackay J**, Parker AC, Leonard RCF. High-dose chemotherapy supported by peripheral blood progenitor cells in poor prognosis metastatic breast cancer - a phase I/II study. Br J of Cancer, 1996, 74: 2013-2017

Gabra H, Cameron DA, Lee LE, **Mackay J**, Leonard RCF. Weekly doxorubicin and continuous infusional 5-fluorouracil for advanced breast cancer. Br J of Cancer, 1996, 74: 2008-2012

**Mackay J** The role of genetic testing in breast cancer by the year 2000 Cancer Treatment Reviews 1997, 23 S13-S22

**Mackay J.** The Li-Fraumeni syndrome CME Journal of Gynaecologic Oncology, 1997, 2: 75-77

White E and **Mackay J.** The management of familial ovarian cancer. Hungarian Journal of Gynaecologic Oncology, 1997, 2: 129-132

Crosbie A, Brewer C, Campbell K and **Mackay J.** BRCA1 gene testing for breast and ovarian cancer in one familyBr J Nursing, 1998 22:1386-92

Pharoah PD and **Mackay J.** Absolute risk of breast cancer in women at increased risk: a more useful clinical measure than relative risk?The Breast, 1998 7, 255-259.

Pharoah PD, Stratton JF and **Mackay J.** Screening for breast and ovarian cancer; the relevance of family history.British Medical Bulletin, 1998, 54 No 4.

Cull A, Miller H, Porterfield T, **Mackay J**, Anderson EDC, Steel CM, Elton RA. The use of videotaped information in Cancer Genetics Counselling. A randomised evaluation studyBr J Cancer, 1998, 77: 830-837

Cull A, Anderson ED, Campbell S, **Mackay J**, Smyth E and Steel M. The impact of genetic counselling about breast cancer risk on women's risk perceptions and levels of distress. Br J of Cancer, 1999. 79 (3-4):501-8.

Leggatt V, **Mackay J**, Yates JWR. Evaluation of a questionnaire on cancer family history in identifying patients at increased genetic risk in general practice.BMJ. 1999; 319: 757-758.

Thomas R, Thornton H and **Mackay J.** Patient information materials in oncology – are they needed and do they work? Clin Onc 1999 11:225-231

White E and **Mackay J.** A critical evaluation of the process of cancer genetic counselling: from research based investigation to clinical diagnosis.European Journal of Oncology Nursing, 1999. 3(2):96-101.

Leggatt V, **Mackay J**, Marteau TM, Yates JRW. The psychosocial impact of a cancer family questionnaire completed in general practice. J Med Genetics, 2000 37:470-472.

Eccles DM, Evans DER and **Mackay J.** Guidelines for a genetic risk based approach to advising women with a family history of breast cancer.J Med Genetics, 2000 37:203-209.

Crawford R, Everest S and **Mackay J.** Familial Ovarian Malignancy.Hospital Medicine 2000 61: 528-531.

Broadstock M, Michie S, Gray J, **Mackay J**, Marteau T. The psychological consequences of offering mutation searching in the family for those at risk of hereditary breast and ovarian cancer – a pilot study.Psycho-Onc 2000; 9:537-548.

Watson EK, Clements A, Yudkin P, Rose P, Bukach C, **Mackay J**, Lucassen A and Austoker J. Evaluation of the impact of two educational interventions on GP management of familial breast/ovarian cancer cases: a cluster randomised controlled trial.British Journal of General Practice. 2001;51:817-821.

Hyland F, Kinmonth AL, Marteau TM, Griffin S, Murrell P, Spiegelhalter D, Todd C, Walter F, Berrington B, Bobrow M and **Mackay J** - Women's Concerns Study Group Raising concerns about family history of breast cancer in primary care consultations: prospective, population based study.BMJ, 2001: 322, 27-28.

O'Driscoll D, Warren R, **Mackay J**, Britton P and Day NE. Screening with breast ultrasound in a population at moderate risk due to family history.J Med Screen. 2001; 8:106-109

**Mackay J**, Rogers C, Fielder H, Blamey R, Boggis C, Brown J, Pharoah P, Moss S, Day N, Myles J, Austoker J, Gray J, MacMillan D, Cuzick J and Duffy SW. Development of a protocol for evaluation of mammographic surveillance services in women under 50 with a family history of breast cancer.J Epidem. Biostats. 2001; 6: 365-369

Hallowell N, Jacobs I, Richards M, **Mackay J** and Gore M. Surveillance or surgery? A description of the factors that influence high-risk premenopausal women's decisions about prophylactic oophorectomyJ Med Genet 2001;38:683-726

Watson E, Clements A, Lucassen A, Yudkin P, **Mackay J** and Austoker J. Education improves GP management of familial breast/ovarian cancer: findings from a cluster randomised controlled trial.J Med Genet 2002: 39:779-781

Foster C, Evans DGR, Eeles R, Eccles D, Ashley S, Brooks L, Davidson R, **Mackay J**, Morrison PJ and Watson M. Predictive testing for BRCA1/2: attributes, risk perception and management in a multi-centre clinical cohort.Br J of Cancer 2002: 8: 1209-1216

Hopkinson I and **Mackay J**. The implications of the Human Genome project; inherited variations in cancer care. Annals of Oncology 2002: 13: Suppl. 4 81-87

IBIS investigators. First results from the International Breast Cancer Intervention Study (IBIS-1) – a randomised prevention trial.Lancet 2002: 360: 817-824

Andermann A, Austoker J, Watson E, Lucassen A, **Mackay J**. Development and evaluation of a general information leaflet for women with family history of breast cancer.Journal Cancer Education. 2002:17:155-160

Richards M, Ponder M, Everest S, Pharoah P and **Mackay J**. Issues of consent and feedback in a genetic epidemiological study of women with breast cancer. Journal of Medical Ethics, 2003:29:93-96

Wallace E, Hinds A, Campbell H, **Mackay J**, Cetnarskyj R and Porteous M. A cross sectional survey to estimate the prevalence of family history of colorectal, breast and ovarian cancer in a general practice population.Br J of Cancer; 2004; 91: 1575-1579.

Hallowell N, **Mackay J**, Richards M, Gore M, Jacobs I. High-risk premenopausal women's experiences of undergoing prophylactic oophorectomy: a descriptive study.Genet. Test. 2004. 8: 148-156

Watson M, Foster C, Eeles R, Eccles D, Ashley S, Davidson R, **Mackay J** et al. Psychosocial impact of breast/ovarian (BRCA1/2) cancer-predictive genetic testing in a UK multi-centre clinical Cohort.Br J. of Cancer; 2004. 91: 1787-1794

Braithwaite D, Sutton S, **Mackay J**, Stein J and Emery J. Development of a risk assessment tool for women with a family history of breast cancer: a randomised pilot study; Cancer Detection and Prevention; Volume 29, Issue 5 , 2005, Pages 433-439.

Pithers A and **Mackay J**. Cancer genetics – an overview of an information project. European Journal of Cancer Care; 2005. 14, 188-197.

Coelho JJ, Arnold A, Tischkowitz M and **Mackay J**. Assessment of the efficacy of cancer genetic counselling by telemedicine compared to face-to-face counselling.European Journal of Cancer; Eur J Cancer. 2005; 41(15):2257-61.

Kalra D, Singleton P, Milan J, **Mackay J**, Detmer D, Rector A, Ingram D
Security and confidentiality approach for the clinical E-science framework (CLEF)
Methods Inf Med 2005. 2, 193-197

**Brett J, Bankhead C, Henderson B, Watson E, Austoker J.** The psychological impact of mammographic screening: A systematic review. Psychooncology. 2005 Nov; 14(11):917-38

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Date: 18th September 2008

Signature:

James Mackay

## Examination Guidelines

### Guidelines as to relevant knowledge and experience for sitting Advanced Level Paper P2

### Introduction

These guidelines have been prepared to assist candidates in preparing for the examinations. They are guidelines only and do not indicate a level of knowledge or experience that is required before a candidates can sit a given paper – eligibility for each paper is governed by the examination regulations. Instead the guidelines are intended to be used to identify knowledge and experience that candidates should ideally have attained prior to sitting the examination.

It is appreciated that different candidates will have widely different opportunities for training and gaining experience, depending on the pattern of work in their firms or companies. Using these guidelines will help candidates to identify areas where the knowledge and experience gained in the workplace will have to be supplemented through tutorials, internal or external to the workplace, through seminars, training courses, private study or otherwise.

It is considered unlikely that candidates with less than 3 years' experience in the profession, including at least a year acting mainly on their own responsibility, will have sufficient experience to address this paper.

These guidelines have been prepared by a group made up of representatives of ITMA, CIPA, TMPDF and Queen Mary College, University of London as part of ITMA's and CIPA's response to recommendations in the Sherr Report, and updated by the Informals CIPA Education Committee representatives, July 2008 .

### P2 Paper

P2 is a test of the competence to properly advise and act for a client in any matter relating to the preparation, prosecution, exploitation and enforcement of patent rights in the UK and via the EPC and PCT. Knowledge of the basic patent law in major countries (notably the USA and Japan) is also required.

While the precise materials may change from year to year, typically the paper is made up of six compulsory short questions relating to application and prosecution procedural matters and two or three longer questions where a client requires advice on patent matters in a commercial situation. Knowledge of the principles of leading case law is recommended, though it is not necessary to quote any cases. Candidates should be familiar with the different needs and expectations of clients from different backgrounds, e.g. with regards to the consequences and costs of suggested actions, and should give sound practical advice accordingly.

The time allowed for the P2 paper is 4 hours.

**P2 Guidelines**

It is recommended the candidate be familiar with the statutes set out in the syllabus. In addition, it would be helpful for the candidates to have sufficient practical experience, as detailed below:

Miscellaneous Practice Matters

Preferably, candidates should have advised on and/or prepared at least half of the items in the following list, and should be at least aware of considerations for the others in practice:

For GB patents/applications

- Assignment.
- Change of name or address.
- Requesting file wrappers and/or UKIPO register extracts
- Filing statutory declaration or affidavit in support of ex-parte and inter-partes proceedings.
- Request for a discretionary extension of time under r.108 and/or r.110.
- Application to restore a UK patent lapsed for non-payment of a renewal fee.
- Pre-litigation negotiations with a third party.

For EP(UK) patents/applications

- Completing acceptance/grant procedures before the European Patent Office, being thoroughly familiar with key due dates
- Effecting validation of a granted European Patent (UK), being thoroughly familiar with key due dates including renewal fee due dates
- Applying for further processing of a European patent application
- EPO Opposition procedure
- EPO central limitation procedure

For GB or EP(UK) patents/applications

- Opinion on potential protection available for a proposal (not yet the subject of a patent application or other registered protection) in light of the prior art.
- Opinion on inventorship and/or ownership, including employee's rights:
- Opinion on infringement.
- Opinion on validity (patentability).
- Opinion on validity (sufficiency).
- Opinion on availability of convention priority, including requesting a "late" priority claim.

General/Multi-jurisdiction

- Estimate of the cost of filing a UK, European and/or International patent application.
- Taking over representation of a UK/European/International application or granted patent
- Estimate of the cost of filing a patent application in the US or Japan.
- Any matter involving competition law issues.
- Availability of different types of IP right available for a project (e.g. patent, utility model, trade mark, registered and unregistered design rights, copyright, etc.).
- Comparison of claim types available in different jurisdictions.

Patent Office Hearings

Have prepared for, either as principal or assistant:

- 2 ex-parte or inter-partes hearings, at the UK, European or any other national Patent Office.

Have attended, either as principal or assistant:

- 1 ex-parte or inter-partes hearing, at the UK, European or any other national Patent Office

Official Actions from Patent Offices

Have reviewed and advised on:

- 5 official actions from WIPO or a National Patent Office acting as Receiving Office, International Search Authority or International Examining Authority for an International Patent Application.

Have advised on and responded to:

- 10 official actions from the UK and/or European Patent Office, of which at least 5 are from the UK Patent Office.
- 5 office actions from any other Patent Offices, but especially from the USPTO and JPO.

Filing of New Patent Applications

Have filed or sent instructions for filing:

- 10 UK or European patent applications.
- 5 International patent applications.
- 5 patent applications, or 5 national phase entries, at other Patent Offices.
- a national phase entry to the UK national phase
- at least one entry to the European regional phase

Experience external to the workplace

Have attended:

- 12 hours per year of CPD seminars, including at least some outside the workplace.

Have regularly read:

- 1 monthly IP law publication

**Feedback from Examiners**

Examiners' comments for each past paper are available from the Institutes. In addition, a survey of examiners was carried out in 2004.

A summary of the feedback is:-

Examiners find that as far as the knowledge of the Candidates is concerned, there are few areas of particular weakness.

Candidates would benefit by giving more attention to:-

- Priority
- Ownership (who owns rights; how transferred; assignments, etc)
- Novelty (especially 'Article 54(3) EPC' type situations)
- Inventive step
- Imagining being in front of the client and giving advice
- Providing the client with advice, not a list of the law that is relevant
- Identifying problem areas in a question and applying the law to those areas
- Creating a time line where the chronology of events is important to the question

Issues raised in the Examiners' comments one year are often repeated by Candidates the next year. Instead, Candidates should apply the law to the new facts of the question of the examination.

**CIPA/ITMA Short Term WG – February 2005**
**Updated by CIPA Education Committee – July 2008**

# ANNEX 2

Newman, 1996, Radiologic Technology, Volume 68, No. 1, p39-64

# Epidemiology, Diagnosis and Treatment of Prostate Cancer

JULLIANA NEWMAN, B.A., E.L.S.

*Prostate cancer is the most commonly diagnosed cancer in the United States. This article outlines the anatomy of the prostate gland, the epidemiology and natural history of prostate cancer, current diagnostic and screening techniques, treatment options and prognostic implications.*

*This article is a Directed Reading. See the quiz at conclusion.*

The incidence of prostate cancer has increased dramatically during the past 20 years, with an almost 100% increase in new cases during 1994 alone. In 1995, 224,000 new cases were diagnosed — an increase of 44,000 from 1994 — and 40,400 deaths were attributed to prostate cancer.

In 1996, 317,100 new cases of the disease are expected to be diagnosed, resulting in the deaths of 41,400 men and making prostate cancer the most commonly diagnosed cancer in the United States. Breast cancer is anticipated to be the second most commonly diagnosed cancer in 1996, with 185,700 new cases resulting in 44,560 deaths.[1,2]

Prostate cancer tends to affect older men, with most cases occurring in men older than 65. The lifelong probability of dying from prostate cancer is 2.5% to 3% for American men. However, the incidence of prostate cancer in African American men is as much as 30% higher than for their white male counterparts. Furthermore, a recent study suggests that African American men newly diagnosed with prostate cancer tend to have higher levels of prostate-specific antigen (PSA) and larger tumors than white men, even though their cancers are at the same stage. The mortality rate among African American men with prostate cancer is double that of white men. In addition, African American men tend to be diagnosed at a later stage of disease and may suffer from higher mortality rates as a result.[3]

Patients diagnosed with prostate cancer because of symptoms such as bone pain or urinary difficulties have a much higher mortality rate and are considered incurable. Palliative treatments such as hormonal treatment or chemotherapy are available but are limited in their success.[3,4]

The increased incidence in new cases of prostate cancer is attributed to increased public awareness and the availability of more sensitive screening techniques. The development of the PSA test has greatly enhanced the detection of nonpalpable tumors in asymptomatic patients. In addition, there seems to be some evidence to indicate that prostate cancer may be a heritable disease. Recent studies indicate that men with a first-degree relative who has prostate cancer have a greater risk of developing the disease, a likelihood that increases exponentially with the number of affected relatives.[5]

As many as 30% of men older than 50 have histologic evidence of prostate cancer upon autopsy. It is believed that most men die with prostate cancer rather than die because of it. In general, prostate cancer is a slow-growing disease that has a long doubling time — the time it takes for a tumor to double in size.

Treatment methods range from radical prostatectomy (removal of the prostate and surrounding tissue) to radiation therapy and chemotherapy. Another method is deferred treatment or "watchful waiting," where no treatment is used until there is evidence that the cancer volume has increased and is beginning to spread.[5-9]

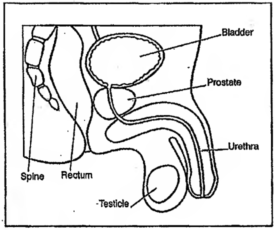


**Fig. 1.** *Cross-sectional illustration of normal male pelvic region. (Artwork reprinted with permission from the American Prostate Society Inc.)*

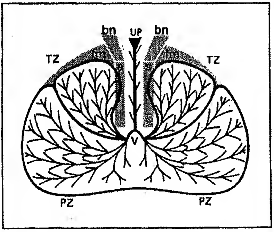


**Fig. 2.** *Oblique coronal section diagram of the prostate, as viewed from the rectal surface, showing location of the peripheral zone (PZ) and transitional zone (TZ) in relation to the proximal urethral segment (UP), verumontanum (V), preprostatic sphincter (s), bladder neck (bn) and periurethral regions with periurethral glands. The anterior fibromuscular stroma (fm) is shown as shaded area. (Artwork reprinted with permission from Dawson NA, Vogelzang NJ.* Prostate Cancer. *New York, NY: Wiley-Liss Inc; 1994.)*

Controversy exists regarding the optimal treatment for patients with early stage prostatic cancer. Due to a paucity of clinically controlled studies, there is insufficient evidence to identify optimal treatment for particular patients. At present, treatment regimens are chosen after careful consideration of the extent of the disease and the patient's preference. Results from prostatectomy, radiation therapy and watchful waiting are variable, and no treatment methodology has been proven clinically superior to the others.

Mass screening for prostate cancer is another topic of controversy. Some experts argue that early detection procedures often are inconclusive and that the early diagnosis of patients with asymptomatic prostate cancer has little impact on overall mortality rates, thus causing unnecessary expense and undue anxiety for patients who are basically at little or no risk. However, recent evidence indicates that there are two distinct types of prostate cancer: clinically insignificant cancers with a low morbidity and mortality rate, and clinically aggressive cancers that consist of a large tumor volume, cells demonstrating moderate to poor differentiation, progressive extracapsular spread and a high PSA level, greater or equal to 10 ng/ml.[10-13]

## Prostate Anatomy

The prostate is a male sex gland that resembles a walnut in shape and weighs about 20 gm.[3,7,8,14-16] (See Fig. 1.) It is located immediately inferior to the bladder and anterior to the rectum. It is transversed by the urethra, and the portion of the urethra passing through the prostate is called the prostatic urethra. The prostate is divided into five histologically distinct zones (see Fig. 2):[8]

- The anterior zone.
- The peripheral zone, accounting for 75% of prostatic volume and the area in which prostatic cancer tends to be localized.
- The central zone, where benign prostatic hyperplasia tends to occur most frequently.
- The preprostatic urethra.
- The transitional zone.

The functions of the prostate gland are not well understood. It is comprised of approximately 30 to 50 tubuloalveolar glands that open into the prostatic urethra through 15 to 30 ducts arranged in a radial pattern within a stroma of fibromuscular connective tissue, blood vessels, lymphatic and nerves. The gland produces an alkaline fluid (pH 6.5) that constitutes

approximately 30% of seminal fluid ejaculate, containing calcium, zinc, acid phosphatase, a clotting enzyme and profibrinolysin.[8]

The majority of cancers of the prostate arise in the peripheral zone and do not affect the periurethral areas until the cancer has spread, explaining the lack of symptoms in patients with early prostate cancer. Symptoms generally do not appear until the cancer has spread and affected other organs. Prostatic cancers can be separated into cancers of acinar (the secretory portion of a gland) and proximal duct origin or of distal duct origin. Adenocarcinoma of acinar origin accounts for 98% of all cancers of the prostate. The prostate itself is rarely associated with metastases from other tumors.

## Possible Risk Factors

Investigators have studied several factors to determine their possible role in the development of prostate cancer,[5,15] including age, heredity, hormones, benign prostatic hyperplasia, diet, socioeconomic factors, occupation, smoking, sexual behavior and vasectomy. Following investigation, several of these factors have been ruled out as risks in the development of the disease.

### *Age*

Prostate cancer is primarily a disease of older men, and age is an important risk factor in its development. (See Table 1). According to the National Cancer Institute's Cancer Surveillance, Epidemiology and End Results Program, in 1990 the age-adjusted incidence rate was 22.7 cases per 100,000 men younger than 65 and 884.1 cases for men aged 65 and older. Between 1985 and 1990, the median age at diagnosis for white men was 72, and the median age of death from prostate cancer was 77. For African American men, the median age at diagnosis was 70, and the median age at death was 75.[5,6,17]

The age-adjusted incidence rates of prostate cancer have increased 3.3% per year in the United States, with the most rapid rise among white men. Recent increases in the incidence of prostate cancer among this group may be the result of a selection bias because a disproportionate number of middle- and upper middle-class American men are undergoing routine screening for cancer, and these socioeconomic groups tend to be predominantly white. In addition, the availability of early detection screening devices is contributing to the proliferation of newly diagnosed cases.

**Table 1**
**Numbers of Deaths by Leading Causes, U.S. Men Ages 55 to 74 and 75+, 1990**

| Ages 55 to 74 | |
|---|---|
| All causes | 430,713 |
| Heart disease | 152,323 |
| Cancer (other than prostate) | 129,364 |
| Chronic obstructive lung disease | 21,964 |
| Cerebrovascular disease | 18,602 |
| Prostate cancer | 12,423 |
| **Ages 75+** | |
| All causes | 447,303 |
| Heart disease | 173,558 |
| Cancer (other than prostate) | 75,117 |
| Cerebrovascular disease | 33,594 |
| Chronic obstructive lung disease | 25,580 |
| Pneumonia, influenza | 24,897 |
| Prostate cancer | 19,622 |

(Data reprinted with permission from Boring CC, Squires TS, Tong T, et al. Cancer statistics, 1994. CA – A Cancer Journal for Clinicians. 1994;44:7-26.)

Autopsy data indicate that as many as 30% of men 50 years or older dying of other causes have histological evidence of prostate cancer. In addition, 50% to 70% of men have asymptomatic prostate cancer by the age of 75. The risk of having asymptomatic prostate cancer is 22% for men in their 60s and 39% for men in their 70s.[5]

### *Family History / Genetics*

Men with a family history of prostate cancer are at greater risk for developing prostate cancer than men without affected relatives.[5] In fact, prostate cancer has a higher familial component than breast cancer or colon cancer, diseases that are recognized as having a strong genetic component.[17,18] Carter's model of the Mendelian inheritance of prostate cancer indicates that the inherited portion of prostate cancer may account for 43% of early onset cancers (onset at younger than 55 years of age).[19,20] However, heritable factors account for only a small percentage (9%) of all prostate cancers. The risk of a familial component in early onset prostate cancer warrants early screening and careful observation for men with affected relatives.

The familial clustering of prostate cancer may be due to two factors — a heritable genetic risk factor and an environmental carcinogen leading to exposure of family members. In a study by Devesa and colleagues, a segregation analysis approach was employed to investigate the possible heritability of prostate cancer. An autosomal-dominant hypothesis fit well with the distribution data in the families studied, suggesting the possibility that a small percentage of prostate cancers (9%) occur as the result of the presence of a Mendelian autosomal dominant gene with high penetrance.[3,7,8,19-20]

The discovery of oncogenes (tumor-causing genes) and antioncogenes (tumor-suppressing genes) has fueled the search for genes involved in the development of prostate cancer.[19] Experts agree that although it is difficult to separate heritable factors from environmental factors, there does seem to be a heritable aspect to prostate cancer. Several studies indicate a high incidence of prostate cancer among blood relatives of men with prostate cancer. Men with a first-degree relative (father or brother) with prostate cancer have a 2.1 to 2.8 times greater risk of developing prostate cancer than the general population. Men who have a first-degree relative plus an uncle or grandfather with prostate cancer are 6 to 8 times more likely to develop prostate cancer than men without an affected relative.[20]

The idea that cancer might be a heritable disease dates to 1911, when Rous demonstrated that sarcoma could be induced by cell-free infiltrates. Many years later, reverse transcriptase, retroviruses and a gene (*v-src*) responsible for Rous sarcoma was discovered.[8,21] In time, many more genes of similar structure were found in other animals, including humans. It was determined that these genes originated in eukaryotic organisms, a superkingdom of organisms including animalia, characterized by membrane-enclosed nuclei containing chromosomes of DNA, RNA and proteins. It was determined that some of these genes were coded for cellular proteins important in cell growth.[8,22,23]

Hereditary cancers can result from the expression of a single gene or several genes, usually mutated suppressor genes (oncogenes) that predispose the host to an increased susceptibility for a particular cancer to develop. Since data indicate that prostate cancer has a strong familial component, experts theorize that oncogenes may play a role. In general, oncogenes function in a dominant manner, and the presence of a single allele can lead to tumor formation. The concept of tumor-suppressor genes or antioncogenes was developed in the 1970s; these genes are believed to function as recessive genes. Therefore, each gene must contain a matching pair of alleles to thwart cancerous activity.[8,24]

Since the early 1990s, more than 60 oncogenes have been identified in the development of certain neoplasms.[25] The normal genetic sequence that provides coding for the manufacture of a cellular proteln is called a proto-oncogene, and its altered or mutated version is called an oncogene. Proto-oncogenes are important in normal cellular growth, encoding for the synthesis of a variety of proteins critical in cell growth as regulators of DNA synthesis, RNA transcriptase and other cellular components. An alteration or mutation in the coding for protein development can cause abnormal growth and contribute to the development of neoplasms. Changes from a proto-oncogene to an oncogene can occur in several ways. The three most common are:[8,20,26,27]

- *Base substitution.* In base substitution, there may be a point mutation in the gene sequence. The prototype for this mutation is the *ras* oncogene, implicated in the formation of a variety of human tumors including urologic neoplasms. Single base mutations at characteristic areas of the gene sequence convert the *ras* gene to an oncogene.[8]
- *Amplification.* A second mutation can occur in which extra copies of a normal DNA gene sequence convert a proto-oncogene to an oncogene. Prototypes of this type of mutation are called amplification, and they include the genes *N-myc*, implicated in neuroblastoma, and *c-erb-B-2*, related to breast cancer.[8]
- *Translocation.* A third type of mutation occurs when an oncogene is moved to a different part of the same chromosome or to a different chromosome. This translocation can result in the inappropriate or overexpression of that gene. The gene also may fuse with another gene, creating a hybrid oncogene. This phenomenon is known as translocational rearrangement and is the prototype for the *c-abl* gene found in leukemia.[8,28]

The search for an oncogene correlated with the development of prostate cancer is ongoing. To date, no oncogene has been directly implicated in the initiation or spread of prostate cancer. The role of several of the growth factor oncogenes, including *ras*, *myc* and *c-erc-B-2*, has been investigated in the development of prostate cancer.[29] Research shows that mutations in the *ras* gene, found commonly in other human tumors, are

rare in prostate tumors.[26,27] Clinical studies reveal a low incidence of *ras* oncogenes in American men with prostate cancer,[30-34] whereas a Japanese study reported the presence of *ras* oncogenes in 25% of Japanese men diagnosed with prostate cancer. The conflicting data may be indicative of potential false-positive results in the Japanese population or of more advanced disease among the Japanese cohort that may have been underrepresented in the American study.[8,34-38]

In addition, the *myc* family of oncogenes has received attention regarding its possible role in the development of prostate cancer. Several studies indicated elevated levels of *c-myc* messenger RNA in prostate cancer compared with benign prostatic hyperplasia (BPH) and normal tissue, a tendency that seemed to increase with higher grade tumors.[37-41] However, studies investigating the level of *c-myc* in two well-differentiated tumors found similar levels in adjacent nonmalignant BPH tissue. Based on this information, investigators concluded that *c-myc* expression is not common in prostate cancer. However, in a mouse prostate model, the combination of *ras* plus *myc* induced predominantly malignant carcinomas.[42]

Research indicates that the *c-erb-B-2* oncogene implicated in breast cancer may play a role in the development of prostate cancer. However, more research is needed to answer this question.

Several other oncogenes have been investigated to determine their possible roles in the development of prostate cancer. Oncogenes *Bcl-2* and *c-kit* may have some role in certain types of prostate cancer. *Bcl-2* is undetectable in androgen-dependent prostate cancer, but the vast majority of androgen-independent cases exhibited high diffuse expression. As such, researchers are investigating whether *Bcl-2* might be involved in the transition from androgen-dependent to hormone-refractory prostate cancer. Much research is needed, particularly to determine if genetic markers may be useful in distinguishing clinically insignificant cancers from aggressive neoplasms.[8,43,44]

Recent data also suggest that DNA methylation may be important in the regulation of genes involved in tumor and growth suppression. Methylation plays an important role in the regulation of gene expression. It is responsible for the maintenance of X-chromosome inactivation in the female and is implicated in genomic imprinting (differential allele-specific expression). An imbalance in DNA methylation occurs in prostate cancer cells, and it is postulated that this imbalance may result in an underlying genetic instability related to tumor progression. In fact, a recent study by Lee and colleagues identified hypermethylation of the GST$\pi$ gene in 20 out of 20 prostate cancer DNAs examined. This hypermethylation was not found in any DNA samples from the control group of normal or hyperplasic prostate tissue samples. To date, hypermethylation of this gene is the most common genetic alteration noted in prostate cancer. Obviously, continued research is required to further investigate the role of methylation in the development of prostate cancer.[19,20]

*Hormones*

Prostate growth and function are regulated by testosterone and dihydrotestosterone. The testes are the major supplier of testosterone, with the adrenal glands producing approximately 10% of circulating testosterone. Because most prostate cancers demonstrate some degree of androgen-dependence, there has been speculation that hormones may be linked to its development. The fact that eunuchs do not develop prostate cancer and that prostate cancer can be induced in rats by chronic administration of estrogen and testosterone have fueled this notion. Although high circulating levels of testosterone have not been consistently found in men with prostate cancer, there does seem to be an increased conversion rate to testosterone in men with prostate cancer. Ongoing research is necessary to clarify the role of hormones in the development, diagnosis and treatment of prostate cancer.[7]

*Benign Prostatic Hyperplasia*

Benign prostatic hyperplasia (BPH) is a noncancerous condition in which an overgrowth of prostate tissue pushes against the urethra and the bladder. It is a common, nonfatal disease of aging. Several retrospective and prospective studies have linked BPH with a 5.1% to 13.5% risk of prostate cancer.[8,45-48] Other studies, however, have found no link.[3,8,12,15,49-51]

Like prostate cancer, BPH is androgen-dependent and more common among older men. However, unlike prostate cancer, BPH usually is found in the central or transitional zone of the prostate, resulting in urinary symptoms that require treatment. Conversely, prostate cancer usually is found in the peripheral zone and is asymptomatic until there has been extracapsular spread.

Because treatment for BPH often involves an invasive procedure, the discovery of concomitant prostate cancer may be more likely.[15]

*Socioeconomic Factors*

There is no evidence to indicate that socioeconomic status has any impact on the risk for developing prostate cancer, although differences in the incidence of prostate cancer in certain racial groups may have fueled this misconception. Prostate cancer is more common in African American men than white men, and less common among Asian men than white men.[8,45,58-63]

*Smoking*

There is conflicting information regarding the role of cigarette smoking in the development of prostate cancer, although there does seem to be a slightly higher incidence of prostate cancer reported for smokers as compared with nonsmokers. This may be an artifact, however, that is more indicative of health care behavior among smokers, who are more likely to see a physician and possibly undergo screening for prostate cancer.[3,64]

*Occupation*

Although it has been speculated that certain professions may be linked to an increased incidence of prostate cancer, there is no evidence to support this theory. Researchers have investigated a proposed link between prostate cancer and men working as farmers, mechanics, newspaper personnel, plumbers, welders, electroplate workers and rubber workers. No direct link has been found, although there is some evidence that men exposed to cadmium (welders and electroplate workers) may have an increased risk for prostate cancer.[65-70] Cadmium is a naturally occurring antagonist of zinc, which is necessary in intracellular metabolism and crucial in the production of DNA-repair enzymes.[71,72] Interestingly, the healthy prostate has a higher concentration of zinc than any other organ. Furthermore, normal or hyperplasic prostatic tissue has higher concentrations of zinc than cancerous tissue.[73-78]

*Diet*

Evidence suggests that the consumption of a high-fat diet may be associated with an increased risk of developing prostate cancer. Armstrong and Doll compared data for prostate cancer death rate and average fat consumption in 32 countries and found that countries with high average fat consumption also had a higher death rates from prostate cancer.[8,51] Diets high in animal fat resulted in the increased excretion of androgens and estrogens, believed to play a role in the development of prostate cancer. In contrast, a vegetarian diet has been linked with lower serum testosterone levels. The dietary hypothesis may offer a possible explanation for the increased incidence of prostate cancer reported in men who have emigrated from regions of low incidence, such as Japan, to areas of high incidence, such as the United States.[79-93]

For example, studies investigating the mortality rate from prostate cancer for Japanese men who migrated to Hawaii revealed a continued low mortality rate for approximately one generation, subsequently reaching a higher mortality rate closer to that of the general American male population. Similarly, studies indicate that the incidence of prostate cancer is higher among European immigrants to the United States than their nonimmigrant countrymen.[8,52,79,80,90,92,93]

*Sexual Behavior*

Despite numerous studies, there is no evidence linking sexual behavior, fertility and prostate cancer.[5,8]

*Vasectomy*

Several studies have shown a slight increase in the risk for prostate cancer following a vasectomy. This increased incidence may be the result of a selection bias, however, because men undergoing vasectomies are more likely to be screened for prostate cancer. At this point, any correlation between vasectomy and an increased risk for prostate cancer cannot be determined to be causal and the risk, if any, is small. Further study is required to settle this issue.[3,8]

## Diagnostic Evaluation And Early Screening Techniques

*Digital Rectal Examination*

Digital rectal examination (DRE) is still considered to be one of the mainstays in the diagnosis of prostate cancer. The prostate is similar in consistency to the tip of a human nose. Carcinoma of the prostate generally has a firmer consistency and can be palpated upon digital examination. The American Cancer Society recommends that men 40 years of age or older undergo an annual DRE because of its relatively low cost and ease of administration. It is estimated that 12,000 curable prostate cancers are detected each year with DRE alone. Despite this, there has been much criticism regarding the accuracy and sensitivity of DRE. Results are subjective and contingent upon the skill of the clinician. In addition, many tumors discovered with DRE alone are upstaged at the time of surgery.[3-8,95-97]

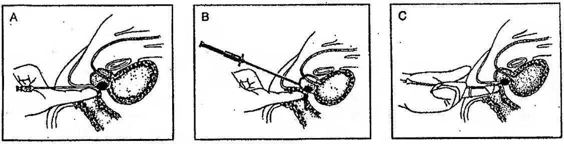


**Fig. 3.** *Comparison of prostate biopsy techniques.* **A:** *Transrectal core needle biopsy.* **B:** *Transperineal core needle biopsy.* **C:** *Fine-needle aspiration biopsy. (Artwork reprinted with permission from Catalona WJ.* Prostate Cancer. *Orlando, Fla: Grune & Stratton Inc; 1984.)*

### *Prostate-specific Antigen*

The discovery of prostate-specific antigen has helped in the early diagnosis of prostate cancer. PSA is a glycoprotein produced and measurable in the cytoplasm of both benign and malignant prostate cells and not found in any other tissues or tumors. PSA levels are directly correlated with tumor size; however, PSA levels can be elevated in men with or without prostate cancer and the level at which PSA should be considered suspicious is controversial. Patients with prostatitis or BPH also may have elevated PSA levels. It is difficult to distinguish prostate cancer from BPH with PSA alone. In general, PSA values greater than 4 ng per mL of blood are considered abnormal. A PSA level between 4.1 ng/mL and 10.0 ng/mL suggests a 1.5 to 3 times above average risk that a man has intracapsular prostate cancer.[3,1,98,99]

Because neither DRE nor PSA are considered definitive in the early detection of prostate cancer, the American Cancer Society and the American Urological Association recommend combining both techniques to more accurately detect asymptomatic prostate cancer. Research suggests that by adding PSA to DRE in a one-time screening program, prostate cancer can be detected in approximately 4.2% of men at age 65 (compared with 2.4% using DRE alone) and in 7.2% of men at age 75 (compared with 3.5% with DRE alone).[38]

Once an abnormal DRE or PSA has been detected, many experts recommend that these patients undergo multiple transrectal needle biopsies, which usually are performed in a single session. Although the procedure may be uncomfortable, it is associated with a low risk of bleeding and related infection.[35,8]

## Radiographic Screening Techniques

Imaging techniques used for the detection of prostatic cancer include transrectal ultrasonography, computed tomography and magnetic resonance imaging.

### *Transrectal Ultrasonography*

Improvements in the spatial resolution of ultrasound has encouraged its use in the detection of disease. Urologists use ultrasound to guide biopsies particularly when lesions that are visible with ultrasound are not palpable. It is well known that the volume of cancer is directly correlated with the probability of extracapsular spread and ultimate prognosis. Although ultrasound has been used to estimate the volume of cancer, it is not very accurate and tends to underestimate the true volume of the cancer. It also has been used in the detection of extracapsular spread of prostatic cancer, but its accuracy in confirming organ-confined cancer is questionable. Furthermore, although preliminary studies suggest that ultrasound is more sensitive than DRE as a method of detecting early prostate cancer, it is an expensive procedure with variable results.[35,8,100]

Transrectal ultrasonography (TRUS) generally is not considered accurate enough to be used as a primary screening test. It has the ability to detect hypoechoic lesions (areas in the ultrasound image where echoes are weaker or fewer in number), consistent with prostate cancer, as small as 5 mm in diameter. It also is valuable in measuring the volume of the tumor and determining its localization. TRUS probes are equipped with a biopsy needle to help document the exact location from which the biopsy was taken. (See Fig. 3.)

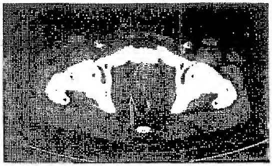

**Fig. 4.** *Extracapsular tumor extension from prostatic cancer visualized by CT. Overall, CT has not been particularly useful in staging local extent of prostatic cancer. (Artwork reprinted with permission from Smith JA, Middleton RG.* Clinical Management of Prostate Cancer. *Chicago, Ill: Year Book Medical Publishers Inc; 1987.)*

A recently developed procedure uses an intermediate-caliber needle for core biopsies using a spring-loaded biopsy gun. No anesthesia is required, and the biopsy gun is used along the finger in the case of palpable lesions or through a biopsy channel built into the TRUS probe. Multiple biopsies are performed in a grid-like pattern to sample possible tumors, even in the absence of identifiable lesions upon ultrasonography.

TRUS can fail to detect as many as 30% of prostatic lesions that are palpable upon DRE because the lesions are isoechoic. Interestingly, while ultrasound can detect small hypoechoic tumors, as many as 80% of these lesions are not cancerous, but rather are indicative of nonmalignant areas of prostatic inflammation.

Not surprisingly, experts disagree about the role of TRUS in the detection of early prostate cancer. It has not been proven that early detection or biopsy of these lesions has any effect on the clinical outcome. Despite the lack of data regarding the clinical utility of TRUS, the procedure has been aggressively marketed to patients in certain regions as a lifesaving technique and is greatly in demand by patients living in those areas.[101]

*Computed Tomography*

Computed tomography is not recommended in the early screening process for prostate cancer; it is generally reserved for screening of biopsy-confirmed cancer to detect cancer spread into the lymph nodes, pelvis, abdomen or spine. CT cannot reveal the internal structure of the lymph nodes, but it is used to determine the size of the lymph nodes as an indicator of neoplasm involvement. However, microscopic disease in the lymph nodes may not result in obvious lymph node enlargement. Therefore, disease may go undetected with CT alone, resulting in a false-negative result. Conversely, lymph node enlargement also may be due to nonmalignant processes such as infection and suggest a false-positive result. For these reasons, CT is not recommended in the early screening process and is reserved for more detailed staging information in biopsy-confirmed cases of prostate cancer. (See Fig. 4.)

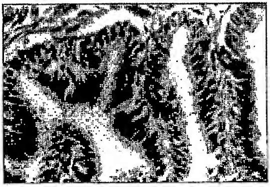

**Fig. 5.** *Normal prostatic acinar epithelium.*

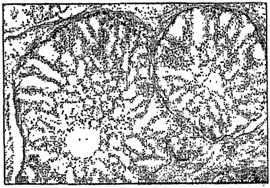

**Fig. 6.** *Cribiform pattern of prostate cancer. (Artwork for both Fig. 5 and Fig. 6 reprinted with permission from Mostofi FK, Sesterhenn I, Sorbin LH. Histological typing of prostate tumors. In:* International Histological Classification of Tumors, XXII. *Geneva: World Health Organization; 1980.)*

*Magnetic Resonance Imaging (MRI)*

Recent studies indicate that magnetic resonance imaging may be useful as a screening device in the early detection of prostatic neoplasms. The use of small probes for transrectal MRI in the detection of prostate cancer have demonstrated high resolution and improved sensitivity in detection. This high sensitivity is critical in distinguishing BPH from carcinoma of the prostate. Appropriate use of this new technology may reduce the need for unnecessary biopsies and improve early detection rates.[102]

## Tumor Grading

Once prostate cancer has been clinically diagnosed using one or a combination of screening techniques, the patient must undergo a staging workup to determine the grade (degree of cellular differentiation between cancerous cells and normal cells) and stage (extent to which the disease is present and has spread). Prognosis and treatment protocols are determined by the grade and stage of the cancer. Several staging systems are used to determine the biological potential of prostate cancer. The predictive accuracy of screening is enhanced by the general correlation that exists between tumor grade and tumor volume. Most small tumors are well differentiated and slow growing, whereas most poorly differentiated tumors are large, aggressive and associated with a poorer prognosis.[3-5,8,103,104]

Normal prostatic epithelium consists of a double layer of simple columnar or pseudostratified cells becoming transitional near the urethra. (See Fig. 5.) In malignant cells there usually is only one layer of cuboidal cells, and a characteristic cribiform pattern may develop. (See Fig. 6.) Another characteristic of prostatic cancer cells is the degree of anaplasia or loss of structural differentiation, such as an increase in the nuclear/cytoplasmic ratio.

*Grading Systems*

Several grading systems have been proposed to help identify distinct histological patterns based on the degree of glandular differentiation. Of the systems available, the Gleason grading system has achieved the widest acceptance. Gleason identified 5 histological patterns based on the degree of cellular differentiation and assigned them values from 1 to 5, with 1 representing well-differentiated cells and 5 representing poorly differentiated cells. (See Fig. 7.) A Gleason score is determined by adding the scores of the two most prominent types of histological patterns. The lowest possible score is a 2, indicating a well-differentiated tumor, and the highest possible score is a 10, indicating a poorly differentiated tumor.[3-5,8,103]

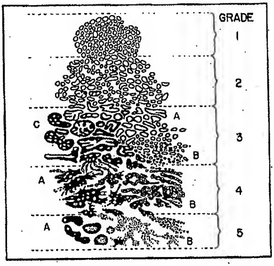


**Fig. 7.** *Gleason system for grading prostate cancer. (Artwork reprinted with permission from Gleason DF. The Veterans Administration Cooperative Urologic Research Group: histologic grading and clinical staging of prostatic carcinoma. In: Tannenbaum M, ed.* Urologic Pathology: The Prostate. *Philadelphia, Pa: Lea & Febiger; 1987:171-198.)*

Well-differentiated tumors, Gleason grades 2 to 5, progress slowly and are associated with a low probability of dying from prostate cancer. (See Fig. 8.) These tumors tend to resemble normal prostate tissue, generally consisting of small, densely packed glands, as compared with poorly differentiated tumors that are characterized by a loss of structural integrity and look like sheets of cells with little tendency to form glands.[104] Patients with poorly differentiated tumors, Gleason grades 7 to 10, have a high probability of dying from prostate cancer. (See Fig. 8.)

The Gleason scale is controversial because of lack of agreement and reproducibility of results. Gleason-trained clinicians tend to agree 80% of the time, whereas non-Gleason trained clinicians agree only 70% of the time. Although the reproducibility of results has been questioned, recent studies indicated that patients

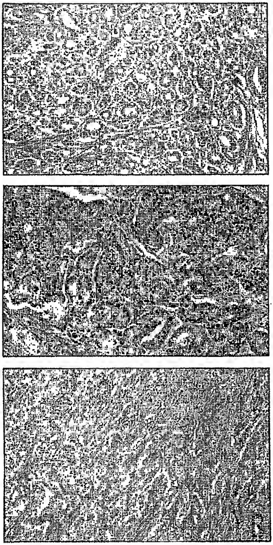

**Fig. 8.** *Different patterns of prostate cancer ranging from well differentiated (top) to moderately differentiated (center) to poorly differentiated (bottom), all occurring in the same tumor. (Artwork reprinted with permission from Mostofi FK, Sesterhenn I, Sorbin LH. Histological typing of prostate tumors. In:* International Histological Classification of Tumors, XXII. *Geneva: World Health Organization; 1980.)*

with a Gleason sum of less than 5 had only a 13% chance of having nodular involvement, whereas patients with a sum of 9 or 10 had a 100% chance of nodal disease.

Another study confirmed these results and showed that a Gleason sum of 8, 9 or 10 was associated with a 93% chance of nodular metastatic disease, and those with a sum of 2, 3 or 4 had no nodal involvement. Patients with intermediate scores of 5, 6 or 7 had a 31% risk of nodal involvement. (See Fig. 9.)

Tumor volume also is an important prognostic indicator in the assessment of extracapsular spread. Tumor volumes of less than 0.5 cc are rarely associated with extracapsular spread. As the tumor volume increases to 1.0 cc and greater, extracapsular spread is common. Tumors greater than 3.0 cm in size usually have invaded the seminal vesicles; overt metastases, however, rarely appear before the tumor has reached 4.0 cm in size.[7]

A study by McNeal and colleagues demonstrated that the volume of the poorly differentiated portion of a moderate to poorly differentiated cancer had a high predictive value for nodal involvement. In the study of 209 patients with prostate cancer, only one of 171 patients with a histologic pattern of 4 or 5 and a tumor volume of less than 3.2 cc had nodular involvement, whereas 22 of 38 patients with a similar histologic pattern and tumor volume greater than 3.2 cc had nodal involvement. Nodal involvement is known to be a poor prognostic indicator, and the risk of dying from prostate cancer is directly proportional to the extent of nodal involvement.[3,6,8,103]

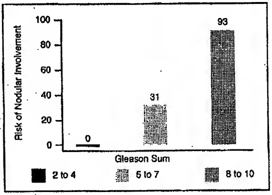


**Fig. 9.** *The risk of nodular involvement as a function of Gleason sum.*

## Tumor Staging Systems

Tumor staging is the careful attempt by the clinician to determine whether cancer has spread from the point of origin and, if so, to what extent. It is essential to an accurate prognosis, and recommended treatment protocols are contingent upon determining the extent of the disease.

Two staging systems are in use: the Whitmore-Jewett system, developed in 1975, and the American Joint Committee for Cancer TNM (tumor, node, metastases) system. The TNM system is gaining popularity.[8]

The Jewett system and the TNM systems are outlined in Table 2. Both systems divide prostate cancer into distinct categories, with subgroups within certain categories. Stage A ($T_{1a\text{-}c}$) tumors are not detectable with DRE. Lesions are generally well differentiated and discovered incidentally. There is no clinical evidence of spread beyond the prostate. Stage $B_1$ ($T_{2a}$) cancer is characterized by moderately to poorly differentiated cells, with a tumor size of less than 1.5 cm and confined to one lobe of the prostate. Stage $B_2$ ($T_{2c}$) cancers are greater than 1.5 cm and may involve two lobes.

When the cancer has spread beyond the prostate, it is defined as Stage C ($T_3$ and $T_4$). Patients often begin to experience urinary abnormalities in this stage. Once metastases have been found in the bones or other organs, prostate cancer is defined as stage D ($T_{1\text{-}4}$, $N_{1\text{-}3}$).

**Table 2**
**Clinical Staging Systems for Prostate Cancer**

| Description | Modified Whitmore | Modified Jewett | TNM (1992) |
|---|---|---|---|
| **Clinically localized disease** | | | |
| Incidental transurethral resection of prostate | A | A | $T_1$ |
| • Focal, low-grade | $A_1$ | $A_1$ | $T_{1a}$ |
| • Diffuse, high-grade | $A_2$ | $A_2$ | $T_{1b}$ |
| Diagnosed on TRUS-guided biopsy, prompted by elevated PSA only | — | — | $T_{1c}$ |
| Clinically detected | | | |
| • Palpable tumor 1 lobe | $B_1$ (≤2 cm)<br>$B_2$ (>2 cm) | $B_{1N}$ (≥1 cm) | $T_{2a}$ (<½ lobe)<br>$T_{2b}$ (>½ lobe but <1 lobe) |
| • Palpable tumor both lobes | $B_3$ | $B_2$ | $T_{2c}$ |
| • Palpable beyond capsule | C | C | $T_{3\text{-}4}$ |
| • Extending to lateral sulcus | $C_1$ | — | $T_3$ (unilateral).<br>$T_3$ (bilateral) |
| • Extending to base of seminal vesicle(s) | $C_2$ | — | $T_3$ |
| • Beyond base of seminal vesicle(s) | $C_3$ | — | — |
| • Invades sphincter, bladder neck or rectum | — | — | $T_4$ |
| • Invades levator muscles or pelvic side wall | — | — | $T_4$ |
| **Metastatic disease** | D | D | $T_{1\text{-}4}N_{1\text{-}3}M_0$ |
| • Pelvic lymph nodes only | $D_1$ | $D_1$ | $T_{1\text{-}4}N_1M_0$ |
| • Bones, lung, etc. | $D_2$ | $D_2$ | $T_{1\text{-}4}N_{1\text{-}3}M_1$ (bone) |
| • Lung, liver, brain | — | — | $T_{1\text{-}4}N_{1\text{-}3}M_{1c}$ |
| • Elevated acid phosphatase only | $D_0$ | $D_0$ | — |
| • Hormonally refractory | Commonly referred to as $D_3$ | | |

### *Stage A Prostate Cancer*

Stage A tumors are referred to as latent or autopsy tumors because they are nonpalpable, produce no symptoms and often are discovered incidentally during treatment for BPH or upon autopsy. (See Fig. 10.) Approximately 10% of patients who undergo removal of the prostate for BPH will have histological evidence of carcinoma upon biopsy. In addition, the clinical prevalence of stage A carcinoma of the prostate is twice the rate of cancers found during prostatectomy in patients of similar age. This phenomenon may help to explain why most stage A carcinomas, which tend to arise in the peripheral zone of the prostate, are underdetected.[3-5,7,8]

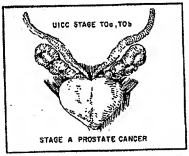


**Fig. 10.** *Digital palpation of the prostate is normal in patients with stage A carcinoma. No nodules or areas of induration suspicious for carcinoma should be evident. (Artwork reprinted with permission from Smith JA, Middleton RG.* Clinical Management of Prostate Cancer. *Chicago, Ill: Year Book Medical Publishers; 1987.)*

The differential progression of stage A tumors has led to the creation of two subcategories: stages $A_1$ and $A_2$. Stage $A_1$ tumors (low grade, low volume tumors) are well differentiated, clinically insignificant tumors that are unlikely to metastasize during the patient's lifetime. In general, low volume is defined as the involvement of 3 to 5 foci or a tumor volume of less than 5% of the total specimen. Stage $A_2$ tumors are considered aggressive cancers. They tend to be diffuse or poorly differentiated and encompass a larger volume than stage $A_1$ cancers, with 6 or more foci or greater than 5% involvement. Stage $A_2$ tumors are associated with distant metastases in as many as 25% of patients. This surpasses the rate of metastases common in patients with $B_1$ tumors and approaches that reported for $B_2$ tumors. These findings underscore the fact that nonpalpable lesions can mask clinically significant cancers, resulting in a poor prognosis and metastatic activity. Stage A cancers usually are detected during transurethral resection of the prostate related to urinary obstruction.

*Stage B Prostate Cancer*

Stage B tumors include all palpable tumors contained within the prostate gland. (See Fig. 11.) Stage B tumors generally are asymptomatic and found during routine examination. There is no evidence of extracapsular spread, and any symptoms of urinary obstruction are related to prostatic hypertrophy. Diagnosis is confirmed with a biopsy of the lesions in question.

Stage B substages are defined according to the size of the palpable lesions. Solitary lesions less than 1.5 cm in diameter are labeled as stage $B_1$ tumors; those over 1.5 cm are considered stage $B_2$ tumors. Tumors with two or more distinct lesions, regardless of size, also are defined as stage $B_2$.[5,6,7,8] Table 3 shows the probability of nodal involvement with stage B prostate cancer.

**Table 3**
**Probability of Nodal Involvement In Patients with Stage B Prostate Cancer**

| | Stage $B_1$ (12% of these pts. have nodal involvement) | Stage $B_2$ (27% of these pts. have nodal involvement) |
|---|---|---|
| % of well-differentiated tumors with nodal involvement / Total % of pts. with well-differentiated tumors | 4% / 33% (1.3% of total) | 18% / 18% (3.2% of total) |
| % of moderately differentiated tumors with nodal involvement / Total % of pts. with moderately differentiated tumors | 14% / 60% (8.4% of total) | 27% / 69% (18.6% of total) |
| % of poorly differentiated tumors with nodal involvement / Total % of pts. with poorly differentiated tumors | 33% / 7% (2.3% of total) | 43% / 13% (5.6% of total) |

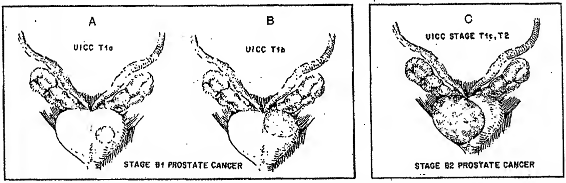


**Fig. 11.** *The two substages of stage B prostate cancer.* **A:** *Stage $B_1$ adenocarcinoma of the prostate. A classic Jewett nodule is less than 1.5 cm in size and surrounded by normal-feeling prostatic tissue.* **B:** *In general, palpable nodules less than 2 cm in size that appear to be confined within the capsule of the prostate are considered to be stage $B_1$ tumors.* **C:** *Stage $B_2$ adenocarcinoma includes tumors greater than 2 cm in size that are confined within the prostatic capsule. In addition, tumors that have two distinct nodules are considered to be stage $B_2$, even if neither is larger than 1.5 cm to 2 cm. (Artwork reprinted with permission from Smith JA, Middleton RG.* Clinical Management of Prostate Cancer. *Chicago, Ill: Year Book Medical Publishers Inc; 1987.)*

*Stage C Prostate Cancer*

Stage C prostate cancer describes locally advanced palpable tumors that have not metastasized to distant areas or to the lymph nodes but have spread beyond the prostatic capsule. Stage C tumors are characterized by lateral spread to the levator muscles or the pelvic side walls or superior spread into the seminal vesicles or base of the bladder. (See Fig. 12.)

Stage C tumors are divided into two substages — $C_1$ and $C_2$. Stage $C_1$ includes tumors with minimal amounts of palpable extracapsular spread, while stage $C_2$ tumors are larger, greater than 5 cm in diameter and may be associated with symptoms or urinary obstruction. Recent studies indicate that stage C tumors account for approximately 15% of tumors found upon initial diagnosis.

Stage C tumors are aggressive, with 70% of patients undergoing disease progression. Only 15% of patients with stage C cancer have well-differentiated tumors; 65% have moderately differentiated tumors and more than 20% have poorly differentiated tumors. Without effective treatment, the 5-year survival rate is 58% and the overall 10-year survival rate is 20%. Seminal vesicle involvement portends a poor long-term prognosis.[55,7,8]

*Stage D Prostate Cancer*

Stage D prostate cancers have metastasized beyond the area surrounding the prostate. Stage $D_0$ identifies patients in whom the only evidence of metastases is an elevated serum acid phosphatase level, which usually indicates extracapsular spread to the lymph nodes or bone. Stage $D_1$ tumors have reached the lymph nodes but are not detected elsewhere. Stage $D_2$ cancers have spread beyond the lymph nodes to other soft tissues and to the bones. Long-term prognosis for patients with stage D tumors is poor, and 75% of patients will develop skeletal metastases in 5 years. Local or regional therapy is ineffective, and many experts believe the patient should be spared the morbidity associated with treatments that are unlikely to offer any benefit.[55,7,8]

## Staging Techniques

Once diagnosed, prostate cancer must be properly staged and evaluated. The volume of prostate cancer is directly correlated with the probability of extracapsular spread and prognosis. Accordingly, the method of treatment is contingent upon the extent of the cancer. Techniques used to determine the extent of dissemination of prostate cancer and histological differentiation include sonography, computed tomography, magnetic resonance imaging, radionuclide bone scans and lymphangiography. The serum level of PSA has been used to stage cancers and according to some experts seems to be the most important predictor of outcome.[14,105]

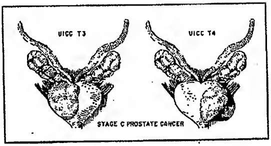


**Fig. 12.** *Stage C prostate cancer includes tumors in which there is extracapsular extension but no distant disease. Tumors often extend superiorly into the base of the seminal vesicle (left) or laterally beyond the prostatic capsule (right). (Artwork reprinted with permission from Smith JA, Middleton RG.* Clinical Management of Prostate Cancer. *Chicago, Ill: Year Book Medical Publishers Inc; 1987.)*

*Computed Tomography*

Computed tomography is used in the local staging of prostate cancer to visualize the prostate, bladder and seminal vesicles and their relationship to other organs. (See Fig. 13.) The evaluation of pelvic lymph nodes is paramount in detecting extracapsular spread and is one of the most important prognostic indicators for patients with prostate cancer.

Unfortunately, however, studies indicate that CT is not very accurate in the assessment of localized prostate cancers, tending to underestimate tumor volume and lymphatic involvement. In fact, most patients with organ-confined cancer will have a normal CT scan. This phenomenon is related in part to the curved nature of the tissue planes surrounding the prostate and seminal vesicles, which are not visualized well in transverse slices. Horizontal tumor extension is hard to detect, and microscopic spread is not visible with CT.

In addition, CT has limited reliability (positive predictability of 70% to 80%) in evaluating the spread of prostate cancer to the lymph nodes. In a study by Gervasi et al,[105] investigators found that for patients with prostate cancer, the risk of metastatic disease at 10 years

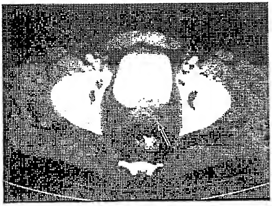

**Fig. 13.** *CT scan confirming stage C cancer of the prostate, indicating tumor extension to bladder base and left seminal vesicle. CT is used in the local staging of prostate cancer to visualize the prostate, bladder and seminal vesicles and their relationship to other organs. (Artwork reprinted with permission from Smith JA, Middleton RG.* Clinical Management of Prostate Cancer. *Chicago, Ill: Year Book Medical Publishers Inc; 1987.)*

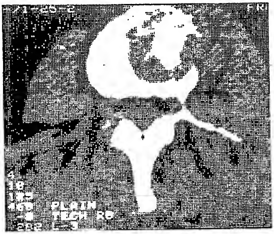

**Fig. 14.** *CT scan showing destruction of left lateral side of vertebral body with soft-tissue mass from metastatic carcinoma of the prostate. CT scans may be particularly useful in determining the nature of abnormal uptake in the spine on a bone scan. (Artwork reprinted with permission from Smith JA, Middleton RG.* Clinical Management of Prostate Cancer. *Chicago, Ill: Year Book Medical Publishers Inc; 1987.)*

was 31% ± 7% for patients with no lymph node involvement, compared with 83% ± 7% for patients with at least one malignant node. Because CT is unable to reveal the internal structure of the lymph node, a diagnosis of extracapsular spread is based on the size of the lymph node as a possible indicator of neoplastic involvement. Since lymph nodes are variable in size, small, nonenlarged lymph nodes with micrometastases may be overlooked and enlarged lymph nodes with nonmalignant processes may be inaccurately diagnosed, making CT an imprecise diagnostic tool for prostate cancer. However, CT scans may be particularly useful in determining the nature of abnormal uptake in the spine on a bone scan.[8] (See Fig. 14.)

*Magnetic Resonance Imaging*

MRI evaluation of the pelvic lymph nodes suffers from many of the same limitations as CT. Diagnosis is based on the relative size of the lymph nodes to determine possible extracapsular spread of the cancer, resulting in false-negative readings when the nodes are within a normal size range and false-positive readings when the nodes are enlarged due to other factors such as unrelated infection. In addition, MRI's sensitivity in the detection of malignant lymph nodes ranges between 4% to 69%, with an average value of 31%.[4,10]

*Lymphangiography*

Bipedal lymphangiography is the most extensively investigated nonsurgical means of evaluating the pelvic lymph nodes for the spread of prostate cancer. It involves the injection of a contrast medium into the lymphatic vessels on the dorsum of the feet and lymph nodes. Radiographs are taken immediately and 24 hours after injection to evaluate lymphatic architecture. Lymph node metastases from prostate cancer may distort the internal structure of the lymph nodes and appear as filling defects within them. An abnormal result occurs when filling defects within the lymph node are identified or when there is gross distortion of the internal organization of the lymph node.

Lymph nodes represent the primary drainage site of the prostate, and there is controversy whether they are consistently filled and visualized during lymphangiography. Due to the profound effects of a false-positive diagnosis, strict criteria of interpretation is employed, reducing the false-positive rate to 10%. In all cases, histological confirmation by fine-needle aspiration should be employed to confirm the diagnosis.[5,6,7,8]

Recently, investigators have combined lymphangiography with fine needle aspiration of filling defects to stage prostate cancer. Biopsies performed of filling defects visualized upon lymphangiography are positive in 50% to 90% of patients with suspicious lymphangiograms, whereas only 15% of patients with normal lymphangiograms have positive biopsies. False-positive results during lymphangiography are uncommon, ranging between 5% and 10%.

Although lymphangiography is associated with a relatively low incidence of false-negative results, it often lacks the sensitivity to detect lymphatic spread. The presence of nodal metastases is detected in only 50% to 60% of patients because some of the nodes are not consistently visualized, and micrometastases cannot be detected. In some cases, extensive metastatic disease can completely replace a lymph node, resulting in nonfilling and lack of detection. In fact, retrospective studies have demonstrated that only 25% of patients with histologically proven lymph node metastases had a preoperative diagnosis of positive lymph node metastases using lymphangiography alone. This statistic underscores the need for a more sensitive and reliable noninvasive staging procedure.[8,10-14]

*Lymphadenectomy*

Lymph node metastases occur in as many as 25% of patients with stage $A_2$ carcinoma, 12% of patients with stage $B_1$ carcinoma, 30% of patients with $B_2$ carcinoma and more than 50% of patients with stage C carcinoma. Stage $D_1$ carcinoma is defined by the presence of nodal metastases.[8-14]

Because noninvasive techniques used to evaluate lymphatic involvement have proven to be less than accurate, many urologists use lymphadenectomy, or dissection of the lymph nodes, to detect the presence of metastases. Lymph node dissection is used as a staging procedure prior to radical prostatectomy to eliminate the possibility of extracapsular spread. Dissection also should include primary areas of prostate drainage to eliminate metastatic activity. The operation originally was designed to remove the obturator and hypogastric lymph nodes and is performed under general, spinal or epidural anesthesia with the patients in the supine or an exaggerated lithotomy position.[106] (See Fig. 15).

Unfortunately, a false-negative rate for lymphadenectomy as high as 8% to 19% has been reported in patients with normal seeming nodes, and frozen section has failed to identify as many as 20% to 40% of patients with microscopic metastases. In addition, there is significant morbidity associated with the procedure, including edema of the lower extremities and genitals and related infection. Combination therapy of lymphadenectomy and external radiation beam therapy is particularly morbid, with as many as 46% of patients reporting disabling edema of the genitals and/or lower extremities.

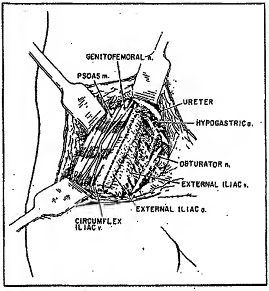


**Fig. 15.** *Pelvic anatomy for modified pelvic lymph node dissection as a staging procedure for prostate cancer. The dotted line highlights the area of the dissection. (Artwork reprinted with permission from Smith JA, Middleton RG.* Clinical Management of Prostate Cancer. *Chicago, Ill: Year Book Medical Publishers Inc; 1987.)*

Recent reports of a modified or limited nodal dissection suggest favorable results. Modified lymphadenectomy seems to be associated with reduced side effects, lower morbidity and less operating time.[106] Controversy exists concerning the sensitivity of the test, however, with experts debating the range of false-negative results.

Tumor grade is also an important indicator of pelvic lymph node metastatic activity. More than half of patients with poorly differentiated tumors at any stage have nodal infiltration, compared with a 10% incidence of nodal spread in patients with well-differentiated

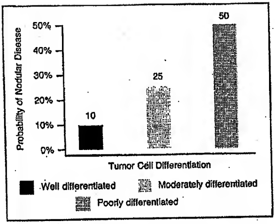


**Fig. 16.** *Correlation between the level of tumor cell differentiation (well differentiated, moderately differentiated or poorly differentiated) and the likelihood of nodular extension.*

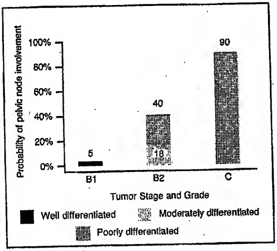


**Fig. 17.** *Correlation between tumor stage and grade and likelihood of pelvic node involvement. Tumor grade and stage are directly correlated with the risk of extracapsular spread. The figure shows that 5% of well-differentiated stage $B_1$ tumors have pelvic node involvement, 18% of moderately differentiated stage $B_2$ tumors have lymph node involvement, 40% of poorly differentiated stage $B_2$ tumors have nodal infiltration, and 90% of poorly differentiated stage C tumors have spread to the lymph node.*

tumors. Approximately 25% of patients with moderately differentiated tumors have nodal involvement. (See Fig. 16.)

Considered in tandem, tumor stage and grade are probable indicators of the status of pelvic lymph node metastases. The incidence of lymph node metastases in patients with well-differentiated stage $B_1$ tumors is less than 5%, whereas 18% of patients with moderately differentiated stage $B_2$ tumors will have metastases. This rate jumps to 40% in patients with poorly differentiated $B_2$ lesions. Not surprisingly, poorly differentiated stage C tumors are associated with a 90% risk of lymph node metastases.[106] (See Fig. 17.)

*Radionuclide Bone Scanning*

Bone scans are the most sensitive means available to detect skeletal metastases and are used to confirm or eliminate a diagnosis of stage D prostate cancer. Approximately 10% to 50% of patients with distant metastases will have positive bone scans despite normal bone radiographs. Solitary lesions may be difficult to interpret, and as many as 10% of metastatic lesions are solitary. Single abnormal areas of uptake are more likely to be due to cancer if located in the spinal column as compared to the ribs. Bone destruction also may be evident if a tumor is present. Healing fractures also can appear as solitary lesions, but these can be eliminated from the diagnosis with a complete clinical history. Areas of degenerative arthritis may be identified, but these areas are usually symmetrical and tend to involve the lumbar spine, sacroiliac joints and acromio-clavicular joints.[7,8,13,14]

Bone scans are highly sensitive in the detection of bone lesions. In fact, false-negative bone scans occur in less than 2% of patients. To date, the most effective radiopharmaceutical agent used in radionuclide bone scanning is technetium 99 methylene diphosphonate. It accumulates in areas of increased bone turnover, has the highest bone/soft tissue ratio and permits imaging 2 hours after injection.

Because $^{99}$Tc is excreted by the kidneys, it is possible to visualize the kidneys, bladder and ureter with a bone scan. Ureteral obstruction related to prostate cancer also may be detected using a bone scan. (See Fig. 18.) Hypersensitivity to $^{99}$Tc is uncommon; in addition, patients are exposed to a relatively low level of radiation, averaging around 1.4 rad. It is recommended, however, that questionable bone scan results be reviewed in conjunction with x-rays to further evaluate

areas of increased isotope uptake. Most bone metastases can be seen on radiographs because of increased bone density and loss of trabecular pattern. However, as a primary screening technique, x-rays are considered to be relatively insensitive since a substantial percentage of bone must be replaced by a tumor before a lesion is visualized. The trabecular pattern often is obscured in prostate cancer, making visualization difficult with x-ray alone.[8]

Bone scans also are useful in determining the origin of pain in patients with prostate cancer. Because of the frequent involvement of the spine in metastatic prostate cancer, nerve compression can radiate cancer pain to distant sites. If the sites show no evidence of metastases, palliative therapy can be directed at point of origin in the spine. Soft tissue metastases can occur in the absence of bone metastases in any area of the body and usually are found upon autopsy in patients with late stage disease.[4,7-9]

*Ultrasound*

Ultrasound is useful in the detection of nodes greater than 2 cm, although nodes that large are generally associated with extracapsular spread and distant metastases. Fine needle aspiration should be performed to eliminate false-positive errors in diagnosis.[107]

## Treatment Options

Finding the most efficacious treatment for prostate cancer is a conundrum. Due to a lack of well controlled, long-term studies, it is impossible to identify a particular mode of therapy that will result in optimal treatment for a particular patient. Treatment depends on the grade and stage of the disease as well as patient preference. No single mode of therapy has been clearly demonstrated to be superior to any other mode of therapy for a particular stage of prostate cancer. In addition, many of the studies investigating treatment outcomes have been retrospective studies subject to selection bias.

With the enhanced sensitivity of new screening techniques such as the PSA test, the incidence of newly diagnosed cases of prostate cancer is increasing at a substantial rate each year. In addition, the aging of the population and decreased morbidity from other age-related diseases has led to and will continue to contribute to an increase in the proportion of elderly men making up the male population. Improved survival in elderly men will contribute to an increased incidence in the number of cases of prostate cancer diagnosed and will result in more deaths from prostate cancer over time.[53]

Because prostate cancer will continue to grow as a major cause of death among men, research is under way to develop enhanced early screening and detection protocols to catch and accurately identify prostate cancer in its early stages when treatment is most effective. At present, there is still controversy regarding the importance of early detection and its ultimate effect on mortality rates. To date, early screening attempts have not had a significant impact on overall morbidity and mortality rates, which continue to climb annually.

Current treatment protocols range from watchful waiting for men with low grade, early stage prostate cancer to radical prostatectomy for capsular-confined prostate cancer to palliative therapy for patients with nodal infiltration and distant metastases. Although

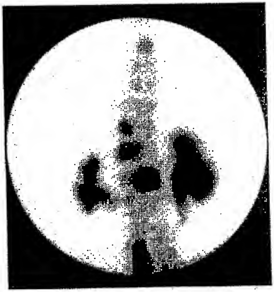

**Fig. 18.** *Bone scan indicating several areas of metastatic uptake in the lumbar spine. In addition, bilateral retention of the radionuclide in the kidneys is evident secondary to ureteral obstruction. Although a bone scan provides relatively imprecise anatomical detail, it can be useful in excluding or detecting uropathy. (Artwork reprinted with permission from Smith JA, Middleton RG.* Clinical Management of Prostate Cancer. *Chicago, Ill: Year Book Medical Publishers Inc; 1987.)*

there is still much confusion about the progression and biologic potential of prostate cancer, several treatment modalities exist that must be individually evaluated regarding side effect potential, recurrence rates, expense and individual preference.

Patients should be presented with appropriate options based on the grade and extent of the disease and counseled regarding a course of therapy. Each treatment method is described below; Table 4 also outlines the current treatment options.

*Surgery (Prostatectomy)*

Radical prostatectomy is the surgical removal of the entire prostate including the capsule, a layer of surrounding connective tissue and attached seminal vesicles. It is recommended when there is no evidence of extracapsular spread, such as stages $A_2$, $B_1$ and $B_2$. The surgery can be performed one of two ways. In retropubic prostatectomy, an incision is made in the abdomen through which the prostate and lymph nodes are removed; in perineal prostatectomy, an incision is made in the area between the scrotum and the anus to remove the prostate. Lymph nodes may be removed through a separate incision in the abdomen.[59,13,14,107]

Evidence of nodal involvement is a contraindication for surgery, and it is therefore essential that patients be correctly staged prior to prostatectomy. Accordingly, a lymphadenectomy is often performed prior to or in conjunction with surgery to confirm the diagnosis of clinically localized cancer and eliminate the possibility of nodal infiltration.

In general, patients treated with radical prostatectomy for clinically localized prostate cancer can expect a survival rate similar to men without cancer in their age cohort. Disease-free survival rates are 91% at 5 years, 47% to 63% at 10 years and 25% to 50% at 15 years. However, direct comparison between survival rates of surgery to other treatment methodologies is not possible due to a lack of available data from controlled studies.

**Table 4**
**Patient Characteristics and Outcomes of Localized Prostate Cancer Treatment**

| | Watchful Waiting | | Radiation Therapy | | Radical Prostatectomy | |
|---|---|---|---|---|---|---|
| | Median (CI) | n | Median (CI) | n | Median (CI) | n |
| **Patient Characteristics** | | | | | | |
| Age | 71 (69 - 73) | 27 | 66 (64 - 66) | 49 | 63 (61 - 64) | 33 |
| Percent of cancers poorly differentiated | 7 (6 - 11) | 19 | 21 (13 - 24) | 45 | 11 (6 - 25) | 22 |
| **Outcomes** | | | | | | |
| Annual mortality rate | | | | | | |
| All causes | .060 (.050 - .04) | 27 | .045 (.040 - .052) | 45 | .032 (.020 - .044) | 27 |
| Cancer-specific | .009 (.006 - .012) | 23 | .023 (.010 - .030) | 22 | .009 (.007 - .013) | 23 |
| Metastatic rate | .017 (.011 - .043) | 15 | .050 (.030 - .095) | 17 | .023 (.014 - .025) | 16 |

KEY: CI = 95% confidence interval; n = number of studies, which varies since not all studies supply all data of interest.

Source: Office of Technology Assessment, 1995. Data from Wasson JH, Cushman CC, Bruskewitz RC, et al. A structured literature review of treatment for localized prostate cancer. Archives of Family Medicine. 1993;2:487-493.

In addition to surgical-related morbidity, radical prostatectomy is associated with a significant possibility of side effects such as incontinence and impotence. (See Table 5.) Urinary incontinence after traditional surgical removal of the prostate occurs in 2.5% to 87% of patients, and permanent impotence occurs in most patients. Both side effects are the result of injury to the bladder opening and the nerve bundles surrounding the prostate that are required in penile erection. Walsh and Donker[108] improved the understanding of prostate anatomy when they discovered the anatomical location of these nerve bundles. (See Fig. 19.) This discovery led to the development of a modified nerve-sparing radical prostatectomy. This modified procedure is associated with less postoperative dysfunction and a substantial reduction in blood loss during surgery. Permanent incontinence occurs in less than 2% of patients, and potency returns within a year for 50% to 80% of patients, although men who have undergone prostatectomy no longer produce semen.[15]

**Table 5**
**Adverse Outcomes of Radical Prostatectomy Among Medicare Beneficiaries**

| Condition | % of Men Reporting |
|---|---|
| Attributable 30-day post-operative mortality | 0.6 |
| Cardiopulmonary complications | 4.0 - 5.0 |
| Incontinence | |
| • Wore pads or other devices for incontinence | 31.0 |
| • Dripped more than a few drops daily | 23.0 |
| • Underwent surgical treatment for incontinence | 6.0 |
| • Had a catheter | 2.0 |
| Impotence | |
| • Had ability to have erections prior to surgery | 90.0 |
| • No full or partial erections since surgery | 61.0 |
| • Had erections firm enough for intercourse in previous month | 11.0 |
| Medical treatment for stricture 2-4 years after surgery | 20.0 |

Source: Office of Technology Assessment 1995. Data from Fowler FJ, Barry MS, Roman A, et al. Patient-reported complications and follow-up treatment after radical prostatectomy: the national Medicare experience, 1988-1990 (updated June 1993). Urology. 1993;42:622-629.

Following radical prostatectomy, PSA levels become undetectable. Because of the high level of accuracy in the detection of PSA, any subsequent increases in PSA levels indicate residual prostatic cancer cells, and a second method of treatment must be considered.[98,99]

*External Beam Radiation*

External beam radiation has proven successful in the treatment of localized prostate cancer and is associated with a success rate comparable to surgery for patients with localized prostate cancer.[3-15,105,109] The survival rate for patients with localized prostate cancer, stages A and B, treated with external beam radiation is similar to that for age-adjusted cohorts without prostate cancer. Local control for stage A and B tumors has been reported in the range of 77% to 97%.

PSA levels also are being used to evaluate the effectiveness of radiation therapy. External beam radiation is the preferred method of treatment for patients with stage C or $D_1$ disease for whom surgery is contraindicated because of extracapsular spread. However, its success rate in the treatment of extracapsular spread remains controversial, and in particular, the combination of prostatectomy and external radiation is extremely morbid and contraindicated. The majority of patients with stage $D_1$ tumors treated with radiation therapy are physically and clinically well for up to a year after treatment, but 69% of these patients will have persistently abnormal PSA levels indicative of residual cancer cells and are at high risk for a clinical relapse.[3-9,98,99]

External radiation generally is delivered in a daily dose of 180 cGy to 200 cGy for a period of 6 to 7 weeks (total dose 6200 cGy to 7400 cGy). Potential complications include impotence, incontinence, cystitis, urethral strictures, prostatitis, diarrhea, edema of the lower extremities and bone marrow suppression. Patients also may become physically tired, and they should be instructed to get as much rest as possible during their course of treatment. It is not uncommon for erythema to develop and for the skin to become red or dry in the area of treatment. Affected skin should be exposed to the air as much as possible and patients should be instructed to avoid wearing tight clothing. Patients also must be instructed not to use creams or lotions on affected skin without their doctor's approval.[14,15]

In addition to traditional external beam photon irradiation, other modalities are being investigated, including protons and neutron beams in an effort to improve delivery and reduce the level of scatter radiation.[3-9]

*Conformal Therapy*

Conformal therapy was developed to enhance the effectiveness of external beam radiation therapy for patients with bulky primary tumors. It usually is reserved for patients with stage $B_2$ and stage C prostate tumors. It also is indicated for patients with persistently positive biopsies after conventional radiation doses of 7000 cGy have not proven effective and larger doses are required for tumor clearance.

Increasing the radiation dose beyond 7000 cGy via conventional radiation is not feasible because of a disproportionate increase in normal tissue complications. Despite the availability of CT and MRI, conventional radiation therapy often is based solely on DRE findings and the use of standard bony landmarks for the establishment of radiation field borders. However, it has been demonstrated that when traditional localization methods are superimposed over CT and MRI images, estimated prostate volume routinely exceeds the volume adequately treated by the small square radiation boost portals.

In an effort to better visualize the prostate and provide higher dose radiation delivery, the technique of conformal therapy was developed.[39,109] Conformal therapy is based on the hypothesis that more successful eradication of tumor cells will be achieved through the application of higher doses of radiation that are conformed to the individual shape and size of the prostate. Accordingly, an individual treatment plan is developed based on a three-dimensional model of the prostate and surrounding organs, factoring tumor localization and volume into account.

By using CT, volumetric imaging is possible from any perspective and treatment can be optimized with respect to tumor coverage and sparing of adjacent tissues. It is possible, in fact, to calculate the exact amount of radiation a given structure is receiving so that areas of under- or overdosing can be identified and corrected during the procedure.[8,109]

Recently, the effect of prostate motion and movement of adjacent organs during external radiation beam therapy has come into question. Because the range of motion of internal organs varies from patient to patient, prostate motion for a particular individual must be taken into consideration when developing a treatment regimen. As such, the target volume required may need to be individualized according to the range of motion.

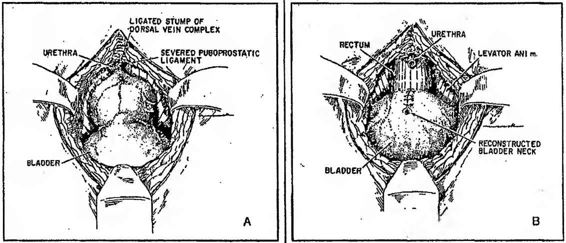


**Fig. 19.** *Anatomical depiction of modified radical prostatectomy.* **A:** *Retropubic exposure of the prostate. The dorsal vein complex has been severed with ligatures. The prostatic apex and urethra are visualized. The neurovascular bundle responsible for erectile function lies posterolateral to the urethra.* **B:** *After the prostate has been removed, bladder neck closure and vesicourethral anastomosis is performed. (Artwork reprinted with permission from Smith JA, Middleton RG.* Clinical Management of Prostate Cancer. *Chicago, Ill: Year Book Medical Publishers Inc; 1987.)*

*Brachytherapy (Internal Radiation Therapy)*

Brachytherapy is the delivery of selective doses of radiation to prostate tumors by the temporary or permanent implantation of radioactive sources. Its clinical appeal has waxed and waned during the past 25 years, with recent advances in visualization techniques and dosimetry models spurring renewed interest.

Developed in the 1970s, the original technique of placing radioactive isotopes into the prostate relied on a relatively imprecise means of localization. Sources were implanted freehand into the gland through an open surgical approach. The advent of a transperineal approach in conjunction with improved imaging techniques permits more accurate visualization of the tumor and has enhanced the accurate placement and distribution of the radioactive sources.[109,110] (See Figs. 20 and 21).

The choice of isotope varies with the stage of the disease. Some of the more commonly used isotopes are iodine 125, gold 198, palladium 103, ytterbium 169, iridium 192 and indium 192. The goal of interstitial radiation therapy (in which radioactive sources are placed within tissue or an organ) is to achieve a specific level of radiation that is sufficient to destroy the cancer cells without harming normal tissue.[8,110]

In terms of dose strength, 7400 cGy to 8040 cGy may be delivered with minimal increases in side effects to normal tissue. CT or TRUS images of the prostate are taken at 0.5 cm increments from top to bottom, and the rectum and bladder neck are identified. Contours of the prostate volume are entered into a treatment planning computer program so that different arrangements of needles and source strengths can be considered. Three-dimensional radiation dose distributions for the prostate, with respect to the rectum and bladder are generated to optimize tumor dose and minimize the radiation dose to normal tissues.[8]

Side effects related to brachytherapy vary according to dose and source used and its placement in relation to other organs and normal tissue. Trauma may be associated with the implantation procedure, such as perineal discomfort, difficulty urinating, hematuria and infection. Hematuria generally resolves itself within 24 hours after implantation. The use of prophylactic antibiotics helps to reduce the possibility of infection. In general, brachytherapy has a favorable side effect profile for patients with localized tumors, although its long-term value relies on its use in the treatment of patients with radiation refractory tumors.[69]

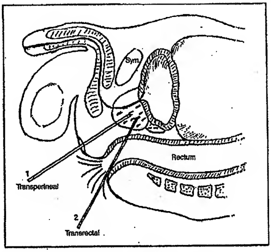


**Fig. 20.** *Transperineal (1) and transrectal (2) needle approach to prostate. Radon seed needles usually are inserted through the perineum, although biopsies can be performed through either approach. (Artwork reprinted with permission from Baumbrucker GO.* Transurethral Prostatectomy: Technique, Hazards, and Pitfalls. *Baltimore, Md: Williams & Wilkins Co; 1968.)*

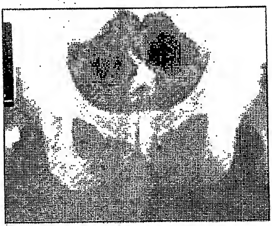

**Fig. 21.** *Radiograph of 14 radon seeds placed in prostate gland using the transperineal route. (Artwork reprinted with permission from Baumbrucker GO.* Transurethral Prostatectomy: Technique, Hazards, and Pitfalls. *Baltimore, Md: Williams & Wilkins Co; 1968.)*

*Glossary*

**Adenocarcinoma** A cancer that develops in the glandular lining of an organ. More than 95% of cancers are adenocarcinomas.

**Adrenal glands** Glands located above the kidneys (one above each kidney). They produce several types of hormones, including a small amount of sex hormones.

**Anaplasia** Loss of structural differentiation, especially seen in most, but not all, malignant cells.

**Androgens** Male sex hormones produced by the testicles and in small amounts by the adrenal glands.

**Antioncogenes** A tumor-suppressing gene involved in controlling cellular growth; deactivation of this type of gene leads to unregulated cell growth as in cancer.

**Autosome** Any chromosome other than a sex chromosome normally occurring in pairs in somatic cells, or singly in gametes.

**Benign prostatic hyperplasia (BPH)** A noncancerous condition in which an overgrowth of prostate tissue pushes against the urethra and the bladder. Also called benign prostatic hypertrophy.

**Lymph** The almost colorless fluid that travels through the lymphatic system and carries cells that help fight infection.

**Lymph nodes** Small, bean-shaped organs located along the channels of the lymphatic system. The lymph nodes store special cells that can trap bacteria or cancer cells traveling through the body in lymph.

**Metastasis** The spread of cancer from one part of the body to another. Cells in the metastatic (secondary) tumor are like those in the original (primary) tumor.

**Oncogenes** Any of a family of genes that normally code for the production of proteins involved in cellular growth and regulation, but that may foster malignant processes if mutated or activated by contact with retroviruses.

**Prostate-specific antigen (PSA)** A protein in the blood that becomes elevated in men with prostate cancer or BPH.

**Prostatectomy** Surgery to remove part of the prostate. A radical prostatectomy is surgery to remove the entire prostate. There are two types of radical prostatectomy: the retropubic prostatectomy (removal of the prostate through an incision in the abdomen) and perineal prostatectomy (removal of the prostate through an incision made between the scrotum and the anus).

**Soma** All of an organism with the exception of the germ cells.

**Transurethral resection of the prostate** The use of an instrument inserted through the penis to remove tissue from the prostate. Also called TUR or TURP.

Internal radiation therapy also is being investigated for its potential use in the palliative treatment of metastatic bone pain. Intravenous injection of bone-seeking radiopharmaceutical agents may be effective in pain control for patients who have not responded to hormonal therapy or local radiation. Response rates ranging from 65% to 80% have been reported with strontium 89 and samarium 153. Side effects include myelotoxicity and an initial flare up of bone pain. This flare-up response may be observed 2 to 3 days post injection and can last as long as 3 to 4 days. Studies suggest that a second injection may be helpful. Clinical trials are being conducted to determine whether this is a viable alternative for pain control in advanced disease.[56,100,110]

Brachytherapy may be performed via permanent implants or temporary, removable implants. Permanent implantation is performed on an outpatient basis with spinal anesthesia. Investigations are under way to develop an improved technique that will increase the accuracy of source placement by using modern treatment planning methods in conjunction with pertinent radiologic data, such as projected tumor doubling time, dose-rate effect and normal tissue toxicity. The incorporation of CT imaging and TRUS permits accurate preimplantation determination of target volume and allows for the precision guidance of trocars inserted percutaneously through the transperineal or transrectal approaches without the need for surgery.[7,8,110]

An example of a permanent implant is iridium 125. It has a half-life of 60 days and delivers radiation at a lower rate relative to other commercially available isotopes. There is concern, however, that tumors with fast doubling rates may reproduce faster than the cells can be eliminated. As such, researchers are investigating the application of new higher-dose radiation sources such as palladium 103 and iridium 192, which may be effective against more aggressive, faster dividing and higher grade tumors. For example, $^{103}$Pd has a half-life of 17 days, and its faster decay rate permits the delivery of a greater hourly dose to higher grade tumors.[7,8,110]

Iridium 192 is used as a temporary, removable interstitial implantation in many types of cancer. This high dose-rate isotope provides radiation ranging from 50 cGy to 80 cGy per hour. Higher dose-rate isotopes pose a greater health threat for personnel exposure during the procedure and require careful handling.

In general, interstitial radiation is used in combination with external radiation for the treatment of more

advanced tumors. Complications vary with the type of implant used and the techniques employed. There is a concomitant increase in the number of potential complications with the use of higher dose-rate isotope implants. The majority of complications involve injury to the urinary tract. However, long-term studies are needed to evaluate the effectiveness of the higher dose-rate isotopes and to effectively compare the clinical outcomes with lower dose-rate isotopes.[7,8,110]

*Chemotherapy*

To date, no truly effective chemotherapeutic agent has been found to halt the spread of metastasized prostate cancer. Most chemotherapeutic agents are used for their palliative effects only and have been ineffective at halting tumor spread.[7,8,110]

*Hyperthermia (Microwave Therapy)*

Hyperthermia, or the therapeutic heating of malignant tissue, has been used successfully to treat benign prostatic hyperplasia in the short term and has not been associated with major complications. Studies indicate that the size of the prostate does not seem to be a factor in clinical outcome and that improvement of symptoms seems to last at least 24 months. Results obtained were similar in scope to those achieved with transurethral resection of the prostate, but without the serious complications possible from surgery.

Hyperthermia using microwave therapy may prove to be a promising alternative to surgery because it can be administered on an outpatient basis, thereby reducing the need for hospitalization, minimizing inconvenience to the patient and reducing related costs.[7,8]

Research shows that heat can cause tissue destruction. Due to tumor vascularity, tumor cells do not dilate in the presence of heat like normal tissue and are more susceptible to the effects of heat damage than normal tissue. Both normal and malignant cells will die at temperatures above 45°C, and both will survive at temperatures below 40°C.

It is important to note that sublethal heating of tumor tissue can result in future resistance to heat treatment. Studies have demonstrated that tumor cells heated to 43°C for 60 minutes will achieve the most pronounced differential. However, the use of hyperthermia in the treatment of prostate cancer is extremely controversial, and there is insufficient clinical experience with this technique to warrant its use outside of a clinical trial.[8]

*Laser Therapy*

Laser ablation of prostate tumors is another application of the use of high temperatures to destroy cancerous tissues, and studies are under way to compare and contrast the use of laser therapy with other traditional treatment methodologies in the treatment of prostate cancer.[7,8]

*Watchful Waiting*

Deferred treatment or "watchful waiting" is a term used to describe a wait-and-see approach to the treatment of patients with asymptomatic, early stage, low-grade tumors. In watchful waiting, no treatment is undertaken until there is evidence that the cancer volume has increased and is beginning to spread. This approach is appealing to many patients because it is the least disruptive course of action.[3-9,13-15,100]

Research indicates that as many as 30% of men older than 50 have histologic evidence of prostate cancer upon autopsy. However, as few as 3% of men diagnosed with prostate cancer will die from it and, in fact, most men die with prostate cancer rather than die from it. These tumors, termed clinically insignificant or autopsy tumors, are believed by some experts to represent a histologically different carcinoma than the more clinically aggressive tumors found in higher-grade prostate cancers. Recently, a group of researchers from the Mayo Clinic defined prostate tumors that are unlikely to grow larger than 20 cc in volume during the projected lifetime of the patient as clinically insignificant tumors.[3-9,13-14]

The best candidates for watchful waiting are patients older than 75; patients with significant comorbidities such as coronary artery disease, insulin-dependent diabetes and chronic pulmonary obstruction; patients with low-grade, low-volume tumors and slow PSA doubling time; and patients with diploid tumors. Short-term survival (5 years) for patients choosing deferred treatment is generally quite high, often more than 90%. Although there is no proof that aggressive therapy for early stage prostate cancer prevents metastases or reduces mortality rates, studies indicate that even with careful monitoring, nearly all of these patients' cancers will eventually progress, with roughly 50% of patients demonstrating evidence of progression 7 years after initial diagnosis. Furthermore, the risk of metastatic spread increases dramatically after 8 to 10 years.[3-9,13-15]

Patients who choose a course of watchful waiting are advised to undergo a yearly DRE and PSA evaluation to monitor cancer growth.[5]

## Conclusion

Although there is much confusion about the progression and biologic potential of prostate cancer, several treatment modalities exist that must be individually evaluated regarding side effect potential, recurrence rates, expense and individual preference. Patients should be presented with appropriate options based on the grade and extent of the disease and counseled regarding a course of therapy.[111-113]

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ANNEX 3

Seiden *et al*, 1994, Journal of Clinical Oncology, Volume 12, No. 12, p2634-2639

# Detection of Circulating Tumor Cells in Men With Localized Prostate Cancer

By Michael V. Seiden, Philip W. Kantoff, Krishna Krithivas, Kathleen Propert, Margaret Bryant, Eric Haltom, Lisa Gaynes, Irving Kaplan, Glen Bubley, William DeWolf, and Jeffrey Sklar

***Purpose:*** **Using prostate-specific antigen (PSA) mRNA as a marker for prostatic epithelial cells, we have developed a sensitive technique that involves reverse transcription and polymerase chain reaction (RT-PCR) to detect circulating tumor cells in the peripheral blood of men with prostatic carcinoma (CaP).**

***Patients and Methods:*** **A sensitive RT-PCR assay was used to evaluate the peripheral blood of 135 men with a history of CaP. Fourteen men with benign prostate disease, many of whom had elevated serum PSA levels, were used as a control group.**

***Results:*** **All patients with benign prostate disease had a negative result in the RT-PCR assay. Of particular interest was a subgroup of 65 patients with clinically localized CaP evaluated before definitive local therapy. Five of these patients had detectable PSA mRNA by RT-PCR, suggesting circulating tumor cells. Within this group, systemic disease was detected by RT-PCR in some men with PSA levels less than 10 ng/mL and clinical stage B disease. Blood from men with hormone-refractory and progressive CaP demonstrated a higher frequency of PSA mRNA detectable by RT-PCR (10 of 20 patients). In contrast, none of seven patients with newly diagnosed metastatic prostate cancer and only one of seven patients with metastatic, hormone-responsive disease had blood that was positive for PSA mRNA by RT-PCR.**

***Conclusion:*** **Circulating tumor cells can be detected in the blood of a subset of patients with clinically localized CaP and a larger subset of patients with progressive metastatic disease.**

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APPROXIMATELY 50% of men with newly discovered prostatic carcinoma (CaP) are diagnosed at a stage of their disease when the tumor is localized to the prostate gland.[1] Local disease control, and potentially cure, can be achieved by either radical prostatectomy or pelvic irradiation in a subset of men with localized CaP. However, both procedures are associated with significant short- and long-term morbidity.[2] Furthermore, staging procedures, currently including physical examination, pelvic computed tomographic (CT) scan, transrectal ultrasound, magnetic resonance imaging (MRI), and bone scan, are imprecise, and understaging is common.[3] Surrogate markers that reflect the potential biologic and clinical aggressiveness of CaP, including histologic Gleason grade and serum prostate-specific antigen (PSA) protein level, may add additional prognostic information to standard staging techniques. Nonetheless, capsular invasion, seminal vesicle invasion, and most importantly, micrometastatic disease are difficult to assess accurately before surgical resection of the prostate. Such understaged patients are less likely to benefit from aggressive local therapy, although they incur all the risk of morbidity associated with local therapy. Therefore, it would be desirable to develop better predictors of occult metastatic disease in patients with clinically localized disease. This report describes initial results of a highly sensitive assay that attempts to identify the subset of patients with clinically localized prostate cancer who have hematogenous dissemination of tumor cells.

Measurement of serum PSA has recently gained a prominent role in the evaluation of patients with CaP. PSA is a 34-kd member of the kallikrein-like serine protease family of proteins.[4] Synthesis of PSA is restricted to prostatic epithelium, and elevated serum PSA protein levels can be found in both benign and malignant diseases of the prostate. Although the serum level of PSA correlates with extent of disease in patients with CaP, accurate prediction of occult metastatic disease in an individual patient is not possible except in cases in which an individual has extreme elevations of serum PSA.[5,6]

As an alternative to serum PSA protein, we have explored PSA mRNA as a potential marker of metastatic CaP. In principle, RNA should have certain advantages over protein as a marker for metastatic disease. First, RNA is very unstable in the extracellular environment; therefore, detection of RNA should suggest the presence of circulating cells that express that particular RNA species. Additionally, sensitive detection of specific mRNA

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species is possible through modification of the polymerase chain reaction (PCR) technique to include a preliminary step of reverse transcription (RT-PCR). With these considerations in mind, and given the limitations of serum PSA as a predictor of metastatic CaP, we prospectively investigated PSA mRNA as a marker for circulating tumor cells in the blood of patients with CaP.

## PATIENTS AND METHODS

### *Patients*

After approval by each institution's human study committees and protocol review boards, patients were invited to participate in this trial at the Dana-Farber Cancer Institute and Beth Israel, New England Deaconess, and Metrowest Hospitals. Patients seeking consultation for either localized or metastatic, treated or untreated CaP were asked to participate, along with a group of men without prostate cancer (control group). Most of the men in the control group had an elevated serum PSA for which they were previously evaluated and found to be free of CaP. Approximately 2.5 to 5 mL of blood was collected in edathamil anticoagulant from each study participant. Blood was drawn before digital rectal examination (DRE) and more than 2 weeks after prostate biopsy in all cases. Patient information, including clinical stage, Gleason grade, and current or past therapy (if any), were collected at the time of entry onto the study. Investigators who performed the RT-PCR assay were blinded to the patients' clinical characteristics, and clinical investigators were blinded to the RT-PCR results. No clinical decisions were made based on the results of this assay.

Patients were divided into three major clinical groups: (1) no cancer; (2) localized CaP; and (3) metastatic CaP. Patients in the no-cancer group consisted of 14 men who had a recent prostate evaluation and were free of CaP. Six of these men had elevated serum PSA (PSA > 4.0 ng/mL); many of the men in this group had undergone random prostate biopsy. Localized CaP was defined by criteria of normal bone scan, normal CT scan, and physical examination findings consistent with disease localized to the prostate or the periprostatic tissue. Patients with localized disease were further subdivided into three groups: (1) patients considered disease-free after local therapy (ie, no measurable serum PSA after radical prostatectomy or, alternatively, normal or decreasing serum PSA after radiation therapy); (2) patients with increasing PSA after definitive local therapy but without evidence of distant metastases; and (3)

**Table 1. Primer Sequences, Their Genomic Position, and Orientation**

| Sequence | Genomic Position | Orientation |
|---|---|---|
| P1 5' TATCGTAGAGCGGGTGTGGG 3' | 3211-192 | Sense |
| P2 5' GTCTGCGGCGGTGTTCTGGT 3' | 1420-40 | Antisense |
| N1 5' TGAAACAGGCTGTGCCGACC 3' | 3149-30 | Sense |
| N2 5' TCCTCACAGCTGCCCACTGC 3' | 1455-74 | Antisense |
| H1 5' GGAACAAAAGCGTGATCTTGC 3' | 1479-82, 3111-27 | Sense |

NOTE. Primers P2 and N2 are complementary to the antisense strand whereas primers P1 and N1 are complementary to the sense strand. Sense and antisense primers are positioned on either side of a 1.6 kilobase intron and do not amplify genomic DNA. The P1-P2 primary PCR product and the N1-N2 nested PCR product are 164 and 64 base pairs in length, respectively.

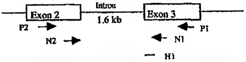


**Fig 1. Schematic representation of a portion of the PSA gene showing the relative position of the specific PCR primers.**

patients with untreated localized disease. Finally, men with metastatic disease were subdivided into three groups: (1) patients with hormone-refractory disease as evidenced by a rising serum PSA after hormone therapy; (2) untreated metastatic disease; and (3) patients with a decreasing or stable serum PSA while on hormonal therapy.

### *RT-PCR*

Preliminary tests with patient samples and the LNCaP tumor line demonstrated that PSA that contained tumor cells copurified in the mononuclear cell fraction (data not shown). Thus, for all samples, the mononuclear cell fraction of peripheral blood was isolated by density gradient centrifugation. RNA was isolated from the mononuclear cell fraction by single-step guanidinium thiocyanate extraction.[7] RNA obtained from mononuclear cells separated from 0.5 mL of blood was reverse transcribed using primer P1 (Fig 1) and modified mouse mammary tumor virus reverse transcriptase, as described by the supplier (Gibco BRL, Gaithersburg, MD). Primer sequences, their genomic position, and orientation are listed in Table 1.[8] cDNA was amplified by two rounds of PCR using nested primers. Primers were designed to span a 1.7-kilobase intron and were selected with the aid of primer analysis software OLIGO 4.0 (National Biosciences, Plymouth, MN). Both rounds of PCR consisted of 35 cycles of DNA amplification. The first round of PCR used 25 pmol of the P1 and P2 primers. Cycle times and temperatures for the primary PCR reaction were 15 seconds at 95° for template denaturation; 30 seconds at 50° for primer annealing; and 30 seconds at 72° for primer extension. The second round of PCR used a 1:50 dilution of the primary PCR reaction and 25 pmol of N1 and N2 primers. The variables for each cycle were 15 seconds at 95°, 30 seconds at 50°, and 30 seconds at 70°. Reactions were performed in 10 mmol/L tris (pH 8.8), 10 mmol/L potassium chloride, 1.5 mmol/L magnesium chloride, 200 nmol of each deoxynucleotide, and 2.5 U of thermophilus aquaticus (Taq) polymerase. PCR reactions were performed in the Perkin Elmer 9600 thermocycler (Emeryville, CA). PCR products were displayed by gel electrophoresis, and their specificity subsequently was confirmed by Southern analysis using a radioactive phosphorus end-labeled hybridization probe (H1) that contained PSA nucleotide sequence (Fig 1).

Integrity of RNA for RT-PCR assay was determined by performing parallel RT-PCR reactions using primers specific for keratin 18 and/or $\beta$-actin mRNA. Samples that failed to yield amplified products for keratin 18 or actin RNA were considered noninformative.

## RESULTS

The LNCaP cell line, a PSA-secreting human CaP cell line adapted to growth in tissue culture,[9] was used to

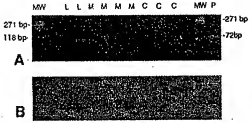


Fig 2. RT-PCR of PSA mRNA. (A) Photograph of an ethidium-stained 2.2% agarose gel showing PCR products from amplification of PSA nucleotide sequences using the nested primers shown in Fig 1. Included in the gel are RT-PCR products from patients with clinically localized disease (L), metastatic disease (M), and control samples from men without CaP (C). The lane marked P represents the amplification from the LNCaP RNA and serves as a positive control for PSA mRNA. Lanes marked MW represent molecular weight standards. (B) PCR Southern blot audioradiogram of the gel shown in panel A.

define the sensitivity of the RT-PCR assay. Dilution experiments of the LNCaP tumor cells into normal whole blood showed that the assay could detect 10 cells diluted in 1 mL of whole blood before ficoll separation of mononuclear cells. Additional experiments showed that 2 pg of LNCaP RNA diluted in 1 $\mu$g of normal bone marrow RNA could be detected after a total of 70 cycles of PCR.

Blood from 156 patients was examined by RT-PCR as previously described. Blood from seven patients could not be fully analyzed because of degraded RNA; complete analyses were performed on the remaining 149 patients. Figure 2 shows a typical result of evaluation of seven patients by RT-PCR. In all cases, a prominent band was visualized after ethidium staining at a position corresponding to a 64–base-pair product. Southern hybridization studies with an internal oligonucleotide probe for the PSA sequence confirmed the identity of this product as amplified PSA mRNA in all cases.

Patients were then categorized into three broad groups: men without CaP, with localized CaP, or with metastatic CaP. Patients with CaP were further divided into clinical subgroups according to treatment history and PSA serology (Table 2). Seventeen of the 135 patients (13%) with CaP had a positive RT-PCR assay. Analysis of the three groups showed that no men with benign prostate disease had a positive RT-PCR assay, whereas six of 100 men with localized CaP and 10 of 34 patients with metastatic CaP were positive by RT-PCR. Table 2 lists the RT-PCR results for each group and subgroup. There was a statistically significant association observed between a positive RT-PCR result and clinical status ($P < .001$, Fisher's exact test).

Table 2. RT-PCR Results by Clinical Stage and Treatment Status

| | | RT-PCR Positive | | RT-PCR Negative | |
|---|---|---|---|---|---|
| | Total | No. | % | No. | % |
| Localized disease (total) | 100 | 6 | 6 | 94 | 94 |
| Untreated | 65 | 5 | | 60 | |
| Treated, no evidence of disease* | 23 | 0 | | 23 | |
| Treated, increasing PSA | 12 | 1 | | 11 | |
| Metastatic disease (totals) | 35 | 11 | 31 | 24 | 69 |
| Before hormonal therapy | 7 | 0 | | 7 | |
| Treated, normal or decreasing PSA | 8 | 1 | 13 | 7 | 87 |
| Treated, hormone-refractory disease | 20 | 10 | 50 | 10 | 50 |
| Controls | | | | | |
| No prostate cancer | 14 | 14 | 100 | 0 | |

NOTE. All samples were performed in duplicate. Control patients had a mean PSA of 2.6 ng/mL.

* See text for a complete description of this patient subgroup.

Five of 65 patients in the subgroup of patients with localized and untreated CaP had positive RT-PCR assays for PSA. Table 3 lists some of the clinical characteristics of the patients with a positive assay compared with those with negative RT-PCR results. Although the size of this subgroup is small, there was no overall correlation between a positive RT-PCR assay and serum PSA level, clinical stage, or Gleason grade. However, patients in the most favorable prognosis (stage A1 or Gleason grade < 5) were all negative by RT-PCR.

In the group of patients with metastatic CaP, the data suggest that patients with hormone-refractory disease are more likely to have a positive RT-PCR assay than patients with newly diagnosed metastatic CaP or patients with

Table 3. Characterization of Men With Localized and Untreated Prostate Cancer

| | RT-PCR Positive | RT-PCR Negative |
|---|---|---|
| Number | 5 | 60 |
| PSA | | |
| Median (ng/mL) | 9.7 | 13.1 |
| Range (ng/mL) | 0.3-207 | 4.5-128 |
| Clinical stage | | |
| A1 | 0 | 7 |
| A2 | 0 | 1 |
| B0 | 1 | 13 |
| B2 | 2 | 20 |
| C | 2 | 8 |
| Gleason grade | | |
| 2-4 | 0 | 10 |
| 5-7 | 4 | 37 |
| 8-10 | 1 | 9 |

NOTE. Patients with clinical stage B0 disease had nonpalpable tumors detected as a result of an elevated serum PSA.[14] Clinical stage and Gleason grade was not available on 6 and 8 of the RT-PCR–negative patients, respectively.

metastatic disease currently responding to hormonal therapy.

## DISCUSSION

PCR is a sensitive technique for the detection of residual tumor cells. The ability of this technique to detect minimal residual disease has so far been applied most extensively to lymphoid and myeloid hematologic malignancies.[10,11] Markers targeted by PCR in these studies have consisted of both DNA and RNA, the latter requiring an initial step of reverse transcription in series with PCR. Reverse transcription and PCR used together in this fashion has been termed the RT-PCR technique. Whether the marker used in a particular test has been DNA or RNA depends on the presence of a tumor-specific nucleotide sequence generated by some type of DNA rearrangement, such as a chromosomal translocation or DNA recombination within antigen-receptor genes.

The PCR-based assay described in the present report uses a different category of marker. This marker is tissue specific rather than tumor specific. Its utility rests on the fact that cells containing the marker are highly unlikely to be present at sites other than the normal site of the tissue in question, except in the cases of metastases. In theory, such a marker could be a protein, a nucleic acid, a lipid, or polysaccharide. The chief advantages of RNA as a marker are the sensitivity with which it can be detected by amplification and the relative instability of extracellular RNA in serum. Recently, RT-PCR has been used to identify circulating tumor cells in small numbers of patients with metastatic melanoma and prostate cancer.[12,13] Because PSA gene transcription is restricted to prostate epithelium, the PSA RT-PCR assay provides a sensitive approach to identify tumor cells in the circulation.

This study surveys patients with localized and metastatic, treated and untreated CaP. The finding that 50% of patients with hormone-refractory CaP are positive for PSA mRNA by RT-PCR compares well with the previously published report on a smaller group of patients.[11] This report extends those findings and examines a population of patients with localized prostate carcinoma. The evaluation of this group of patients by RT-PCR is particularly interesting because appropriate management of this population is complicated by both the imprecise clinical staging techniques and the highly variable natural history observed in patients with localized prostate cancer. That approximately 10% of these patients have a positive RT-PCR assay suggests that the assay may identify a subset of patients with circulating tumor cells. Included among this group with positive RT-PCR assay results are some patients with good prognostic features, including clinical stage B0 disease[14] and/or serum PSA values less than 10 ng/mL.

The clinical significance of a positive RT-PCR assay in patients with clinically localized disease requires further evaluation. Important questions that must be addressed include whether a positive RT-PCR PSA assay result predicts clinical relapse, or more significantly, death from CaP in patients with localized disease; specifically, whether this assay provides prognostic information that is independent of clinical stage, grade, and serum PSA. Currently, we are accruing additional patients and observing all patients prospectively in an attempt to answer some of these questions.

Additionally, certain technical issues need to be analyzed. None of the patients in this study had DRE or prostate biopsy within the 2 weeks before blood sample collection. Several studies have shown that DRE and biopsy have little or no effect on circulating PSA serum protein levels.[15,16] Nevertheless, it is possible that these techniques may cause contamination of the circulation with prostate tumor cells or normal prostate epithelium. Importantly, all of the control patients have had frequent DRE, and some patients have had prostate biopsies for the evaluation of elevated serum PSA levels; none of these patients had a positive RT-PCR assay. Nevertheless, future studies are needed to exclude the possibility that DRE or prostate biopsy leads to systemic contamination with tumor cells.

The RT-PCR assay of PSA mRNA in the blood of men with elevated serum PSA protein levels and no documented malignancy was uniformly negative, which suggests good specificity for this assay. Unfortunately, the assay may not be particularly sensitive because less than half of the patients with metastatic CaP had a positive RT-PCR assay. There are several possible explanations for this finding. Some patients may have no or very few circulating tumor cells. Indeed, it is possible that detection of circulating tumor cells by RT-PCR identifies a particular set of patients at unusually high risk for rapid dissemination and/or death from their disease. Alternatively, circulating cells may not express PSA mRNA transcripts. This is especially true in the group of patients currently responding to hormonal manipulation because PSA RNA expression is decreased with androgen deprivation.[17] In addition, it is possible that patients with high Gleason grade tumors may also be less likely to have circulating tumor cells that express PSA mRNA. Partial degradation of the PSA mRNA might also affect the assay sensitivity, although controls performed on all samples in this study make this unlikely. Finally, blood may not be the best

place to look for occult metastatic CaP. It is critical to determine whether micrometastatic disease is most likely to be detected in the blood, pelvic lymph node, or bone marrow, and which type of biopsy specimen is most likely to predict future symptomatic disease. In this context, it is noteworthy that a recent study has shown that RT-PCR can detect occult metastatic CaP in the lymph nodes of patients with localized disease.[18] Additionally we are investigating methods by which the assay can be made more sensitive.

Furthermore, the biologic relevance of RT-PCR detected circulating tumor cells is unclear. Intravasation of tumor cells into the circulation is just one step in a multistep process that ultimately results in symptomatic metastatic disease. Indeed, animal models have suggested that relatively small tumors can shed millions of cells into the systemic circulation, with only a small proportion of these cells developing into clinically detectable metastatic disease.[19] The lack of information about the biologic potential of circulating CaP cells underscores the importance of not attaching clinical significance to this finding until prospective clinical trials have been completed. Recently, immunocytochemical techniques have been used to detect clinically occult tumor cells in the bone marrow of patients with localized breast cancer.[20,21] Patients with detectable micrometastatic disease have had a higher relapse rate and a lower survival than patients who did not have detectable disease. Although these studies have suggested that micrometastatic disease has prognostic significance, it is notable that many patients with detectable micrometastatic disease have no clinical evidence of metastatic disease after several years of follow-up, and that not all detectable disease may be clinically significant. Unfortunately, similar studies in patients with CaP are not available.

Several groups are currently evaluating the prognostic significance of RT-PCR–detected CaP. In particular, we and other investigators are evaluating the role of this assay in the detection of occult metastatic disease in patients with localized CaP.[22] Recently, Katz et al[22] have demonstrated RT-PCR–detectable circulating tumor cells in 39% of patients with localized prostate cancer who subsequently underwent radical prostatectomy. Correlation of the RT-PCR assay result with final pathologic stage showed a good correlation of RT-PCR positivity with seminal vesicle invasion, capsular invasion, and positive surgical margins. If this assay is predictive of clinically occult metastatic disease, it might be useful in identifying men who are not likely to benefit from local therapy. Second, men with early micrometastatic disease may be useful for the evaluation of novel systemic therapies. A similar rationale has formed the basis of current high-dose chemotherapy trials that involve young women with locally or regionally advanced breast cancer.[23]

The extensive application of the RT-PCR technique to patients with other epithelial malignancies should be possible. Patients with any epithelial malignancy in which local definitive therapy is associated with significant morbidity and/or mortality may benefit from prior examination of blood or other tissues for low levels of tumor cells. Preliminary work with the carcinoembryonic antigen mRNA suggests this may be possible in some gastrointestinal malignancies.[24] Bone marrow or stem-cell preparations from peripheral blood in candidates for autologous bone marrow transplant could also be screened for tumor contamination. Recent reports using keratin 19 as target transcripts for RT-PCR suggest that this should be possible.[25] Furthermore, RT-PCR may be useful in monitoring the effectiveness of adjuvant therapy and may allow the design of new adjuvant therapies, the intensity and duration of which is tailored to the individual patient. The realization of all of these goals will require identification of appropriate tissue-specific RNAs for development of RT-PCR assays applicable to a wider group of cancers. Finally, all of these assays will require correlation to clinical outcomes in large cohorts of patients.

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